Is Gilles de la Tourette's syndrome an autoimmune disease?

Hoekstra, Pieter Jan

Is Gilles de la Tourette's Syndrome an Autoimmune Disease?

Pieter Hoekstra

Georges Gilles de la Tourette *was an individual of great talent, subject to overexcitement and extraordinary activity. He wrote books, research articles, communications, and historical papers in psychiatry and neurology, but was well versed and interested in an even wider range of subjects. Later in his life, a young paranoid woman who was confined to a mental hospital shot him three times while he was in a consulting room. One bullet hit him in the head, and although the bullet was easily removed, it was said he never fully recovered from his injury. Tourette suffered from episodes of depression and mania in his last years and died insane, most likely from syphilis.*

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French neurologist/psychiatrist, born October 30, 1857,
Saint-Gervais-les-Trois-Clochers, France;
died May 26, 1904, Lausanne, Switzerland.

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Introduction

Introduction

Gilles de la Tourette's syndrome is a childhood-onset neuropsychiatric disorder characterized by the presence of both multiple muscle movements and vocalizations, referred to as motor and vocal tics. Tics vary greatly between individuals with regard to severity and frequency. Also, tics show a typical waxing and waning pattern over time within individuals. In addition to tics, many individuals show associated behavioral abnormalities which may include obsessions and compulsions, attention deficits, hyperactivity, impulsivity, and social problems.

Once, tics were considered psychogenic phenomena. Together with nail biting and bed watering, they were grouped among 'child neurotic' features. Psychoanalysts linked motor and vocal tics to the repression of masturbatory urges. Two early authors[1] wrote that tics "always represent an expression of some erotic desire, either the desire for physical gratification of a need for love or affection, or for autoerotic pleasure." According to these authors, eye blinks should be read as symbolic for masturbation. In their view, the child learned early on that a movement attracted attention, which made him willing to tolerate ridicule and scorn, making tics difficult to treat. As a consequence, several forms of psychotherapeutic treatments have been applied to tic disorder patients. Because the involuntary movements were thought to represent the dramatization of a conflict between instinctual desires and inhibitions, the tics themselves were not considered to be the focus of treatment, but rather signs to be read on the way to uncovering repressed psychosocial conflict.[2]

In modern views, psychological explanations for the presence of tics no longer hold. Instead, the genetic background of tic disorders is well-established. Also, much progress has been made in the field of neurochemistry and neuroimaging. Evidence is available about the involvement of the basal ganglia and related cortical structures in Tourette's syndrome. Furthermore, abnormalities in neurotransmitter systems have been detailed. Notwithstanding this progress, the exact pathogenesis of tic disorders remains largely unknown. Some research findings suggest a central role for the involvement of autoimmunity in the pathogenesis of tic disorders. Briefly, these seem to suggest that infections may induce or reinforce tics and associated features in susceptible individuals, possibly through the involvement of abnormal humoral immune responses directed against self-tissue antigens. This possible role of immune dysregulations in the pathogenesis of tic disorders is the central topic of the present thesis.

Chapter 1 gives a review of research findings in favor of the involvement of immune factors in tic disorders, and forms the theoretical framework of the experiments described in this thesis. These experiments can be divided in a cross-sectional (chapters 2 through 7) and a longitudinal part (chapters 8 through 10). Apart from a study which investigates the social-behavioral problems of tic disorder patients in relation to comorbid attention deficit/hyperactivity disorder

and obsessive-compulsive disorder *(chapter 2)*, the first, cross-sectional part focuses on three laboratory assessments which may point to the involvement of autoimmunity. First, a flow cytometry study (*chapter 3*) reports on overexpression of the D8/17 surface marker on B lymphocytes in tic disorder patients, compared to healthy subjects. D8/17 B cell overexpression is a putative susceptibility marker of autoimmune sequelae in the aftermath of streptococcal infections. This is followed by a reanalysis and alternative interpretation of the findings of this flow cytometry study (*chapter 4*) and by a study on D8/17 B cell expression in a contrast group, that is, patients with post-streptococcal arthritis (*chapter 5*). The second laboratory parameter in the cross-sectional part of the experiments constitutes the assessment of serum autoantibodies directed against brain self-antigens, which may be a direct indicator of the involvement of autoimmunity. *Chapter 6* describes the results of this study in tic disorder patients, compared with patients with autistic disorder, patients with obsessive-compulsive disorder, and healthy subjects. The final cross-sectional laboratory measure involves the metabolism of tryptophan through the kynurenine pathway (*chapter 7*). Pro-inflammatory cytokines, most notably interferon-gamma, are known to stimulate the enzyme indoleamine 2,3-dioxygenase, which promotes the breakdown of tryptophan to kynurenine. This may lead to increased kynurenine levels, a well-established marker of immune activation across a wide range of inflammatory conditions.

The longitudinal studies described in this thesis also look at three possible factors. First, the association of infections with exacerbations of tic severity is assessed in *chapter 8*. In contrast, the *following chapter* describes the possible influence of small life events on the changing pattern of tic severity over time. Finally, *chapter 10* describes the effect of a therapeutic intervention, intravenously administered immunoglobulins compared to a placebo condition.

The central question of this thesis is, whether or not immune factors play a role in Tourette's syndrome. An overall discussion of this issue is presented in *chapter 11*, taking into account the various results of the experiments described in this thesis. The next section briefly outlines the main concluding remarks, followed by a summary in English and in Dutch.

References

1. O.E. English & G.H.J. Pearson, Common Neuroses of Children and Adults, New York: W.W. Norton; 1937, p.137.
2. Kushner HI. A cursing brain?: the histories of Tourette syndrome. Cambridge (MA): Harvard University Press; 1999.

Chapter 1

Is Tourette's syndrome an autoimmune disease?

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Abstract

We provide a review of recent research findings which support the involvement of autoimmunity in childhood-onset tic disorders, in particular the presence of antineuronal autoantibodies, D8/17 B lymphocyte overexpression, a marker of chorea associated with streptococcal infection, and possible beneficial effects of immunomodulatory intervention. One of the most controversial areas in this field is the validity of the proposed PANDAS concept. Some researchers have delineated a putatively unique subgroup of patients from the spectrum of illness encompassing Tourette's syndrome and obsessive-compulsive disorder, whose tics and obsessive-compulsive symptoms are shown to arise in response to beta-hemolytic streptococcal infections. They designated it by the term pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections (PANDAS). Herein, we additionally present pros and cons concerning the concept of PANDAS. Finally, recommendations for future research directions are given.

Introduction

Gilles de la Tourette's disorder, or Tourette's syndrome (TS), as it is mostly simply referred to, is a neuropsychiatric disorder, characterized by the presence of both motor and vocal tics. A tic is a sudden, rapid, recurrent, nonrhytmic, stereotyped motor movement or vocalization (DSM-IV). Common examples of motor tics include excessive eye blinking, nose twitching, head jerks, and tensing the abdomen, whereas throat clearing, coughing, and sniffing are the most prevalent vocalizations. Tics typically occur in bouts during the day,[1] whereas the course of tics over a period of months to years often waxes and wanes with regard to their severity.[2] Furthermore, the type of tics in an individual patient is typically changing, with some tics disappearing and new ones appearing in the course of time. With increasing age, however, symptoms tend to decrease in intensity and to show less variation over time regarding both severity and type of tics.[3] The age of onset of tics is mostly between 2 and 15 years with a median of 7.[3] Facial tics are normally the initial symptom. Tics are rather common in childhood, but are most often transient.[4] Males are more commonly affected than females.[5] Movements generally decrease during sleep and may be suppressed for short periods while the patient is awake.[6] Transient tic disorders are estimated to affect at least 5% of children between 7 and 11 years; population studies estimate prevalence rates for full-blown TS between 2.9 and 5.2 cases per 10 000.[5] Transient tic disorder, in which symptoms are required to last less than 1 year, and chronic motor or chronic vocal tic disorder, in which only one type of tics (either motor or vocal) is involved, are all thought to be etiologically closely related to TS, thus making this DSM-IV based subclassification of tics into separate categories rather artificial.

One interesting feature of TS is its well-known association with a wide range of behavioral disorders and psychopathology.[7] Attention deficit/hyperactivity disorder (ADHD) is known to affect 50% of referred patients.[8] Obsessive-compulsive symptoms constitute another common cophenomenon of the spectrum of tic disorders.[9] Finally, many children show significant problems with social functioning.[10]

Currently, tics are diagnosed on clinical grounds alone. Though tic disorders are no longer regarded as psychogenic, the pathogenesis is poorly understood. Circumstantial evidence from neurochemical and imaging studies stresses the importance of basal ganglia and cortico-striato-thalamo-cortical circuits.[11]

A high degree of heritability is characteristic of tic disorders. Several investigators found that the pattern of vertical transmission within families fitted best to a mode of inheritance of TS involving a single autosomal dominant locus with varying penetrance. No such single gene defect has been found though, despite extensive linkage studies covering most of the genome.[12] Associations for markers within certain chromosomal regions have been reported recently.[13]

Our lack of understanding of the pathophysiology of TS and associated phenomena is reflected in the paucity of existing treatment options. They are all purely symptomatic and consist largely of the use of various forms of psychotropic medications, mostly antipsychotic agents.[14] In some individual cases, however,

medication does not show any effect on tic severity, whereas in many other cases, the decrease of tic severity achieved by medication is only marginal and far from sufficient. Besides, many patients suffer from troublesome medication side-effects, such as sedation and weight gain. This unfavorable situation can be illustrated by the fact that many patients, on long term, choose to live without medication, despite the existence of debilitating tics.

What model of autoimmunity may be involved in the pathogenesis of tic disorders?

Over the past decade, a significant amount of research has been conducted on the role of autoimmunity in tic disorders. The proposed model of pathogenesis of tic disorders in this research is analogous to Sydenham's chorea.[15] In genetically predisposed individuals, tics and associated phenomena are thought to arise as a consequence of the immunological response to infections with group A beta-hemolytic streptococci (GABHS). Antibodies directed against the streptococci are hypothesized to cross-react with structures of the central nervous system, subsequently leading to damage to these structures, which eventually results in the emergence of tics and associated features. This mechanism of autoimmunity is supposed to be based on molecular mimicry between host and micro-organism.

What data point towards involvement of autoimmunity?

Several clinical observations led to the hypothesis that Sydenham's chorea might be a model for some types of childhood-onset OCD and tic disorders. First, it had been noted that patients with Sydenham's chorea shared certain behavioral characteristics with patients with OCD and/or tic disorders, such as emotional lability, marked irritability, but also frank obsessive-compulsive symptoms.[16] Second, a substantial number of children with OCD were reported to show choreiform movements or tics.[17] In addition, in some children with OCD and/or tic disorders, an episodic course and/or abrupt onset of their symptoms seemed to be temporally related to signs of GABHS infections.[15] Following these observations, case studies began to appear in the literature in which children with OCD and/or tic disorders were described in whom a temporal relationship between symptom onset or exacerbations, and GABHS or viral infections seemed apparent.[18-20]

Researchers of the National Institute of Mental Health (NIMH) subsequently proposed criteria to identify a putatively unique subgroup of patients from the spectrum of illness encompassing TS and OCD whose tics and obsessive-compulsive symptoms are shown to arise in response to beta-hemolytic streptococcal infections. They designated it by the term pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections (PANDAS),[19]

implicitly suggesting that in non-PANDAS cases autoimmunity would not be involved. This concept is not without controversy, however, as will be addressed later in this review. What percentage of children with TS and/or OCD would meet criteria for PANDAS is unknown.

Thus, clinical observations might point to a role for infections preceding the onset or exacerbations of tic or obsessive-compulsive disorders. Other clues for such a relationship stem from laboratory work. Most importantly, autoantibodies reacting with basal ganglia,[21-26] increased serum levels of streptococcal antibodies,[27-29] as well as the presence of increased binding of an IgM monoclonal antibody to B lymphocytes,[22,30,31] a suggested susceptibility marker for the development of rheumatic fever, have been reported in a majority of patients with OCD and/or tic disorders. Other laboratory hints may come from limited findings on tryptophan metabolism[32-34] and cytokine profiles.[35] Finally, rather fascinating is the reported success of immunomodulatory interventions (plasmapheresis or intravenous immunoglobulins).[36] All these items will be discussed in the next sections.

Studies demonstrating antineuronal antibodies

A possible indicator of autoimmunity is the presence of autoantibodies directed against components of the central nervous system. So far, several research groups reported the increased presence of these antineuronal antibodies in sera from patients with tic and/or OCD, compared to healthy controls.[21-26] Results, however, have been partially conflicting, and relatively high levels of antineuronal staining have been found in sera from healthy control subjects. Kiessling and coworkers[21] were the first to assess antineuronal antibody status in children with recent onset of movement disorders (TS, motor and/or vocal tics, chorea, or choreiform movements), compared with a group of children referred for evaluation of ADHD, behavior disorders, and learning disabilities who did not show signs of a movement disorder. They applied an indirect immunofluorescence technique, with unfixed frozen human caudate nucleus sections as antigenic substrate, using undiluted sera and fluorescein isothiocyanate-labeled secondary antibody directed against human IgG, and found 44% of children with a movement disorder to be strongly positive for antineuronal antibodies. However, also 21% of the control group was strongly positive for antineuronal antibodies. It is unlikely, that the behavioral symptoms of the control group explain the positive antineuronal status in the controls, since two more studies applying the same technique[16,22] also found relatively high levels of antineuronal staining in healthy controls, as will be addressed later. Unfortunately, no subsequent two-fold titrations of positive sera were performed in this study, which could have been used to compare titers between patients and controls.

Previously, in 46% of children with Sydenham's chorea, the presence of antibodies reacting with caudate nuclei neurons had been reported.[37] In that study, however, which applied an indirect immunofluorescence technique similar to that in Kiessling's

study, contrary to the latter study, none of the healthy control children showed evidence of the presence of antineuronal antibodies. In contrast, two additional studies, which, like Kiessling's work, adopted the method of indirect immunofluorescence on human caudate nucleus sections as described by Husby,[37] found relatively high percentages of positive staining using undiluted sera of healthy controls: Swedo and colleagues[16] reported positive antineuronal antibody titers in 50% and Murphy[22] in 24% of healthy control children. Unfortunately, in these studies, no explicit data are available about the presence of intercurrent infectious illness or autoimmune disorders in the control subjects. In both studies, the percentages of positive antineuronal staining were consistently higher for the patient groups, being 91% for children with Sydenham's chorea in Swedo's study[16] and 39% for children with tic disorder and/or OCD in the report of Murphy.[22] However, again, a comparison of titers between diseased children and controls could not be made, as serum dilutions were not performed.

To summarize, a major problem with previous studies reporting high levels of antineuronal antibodies in patients with tic disorders is the relatively high level of, possibly, nonspecific antineuronal staining in normal controls. Therefore, it would have been important, if, in both patients and comparison subjects, also staining of antibodies to other brain regions than the caudate nucleus would have been assessed, in order to control for nonspecific antineuronal staining. A recent study, also applying indirect immunofluorescence with unfixed frozen caudate sections, using undiluted sera and fluorescein isothiocyanate-labeled anti-human IgG as secondary antibody, was, however, able to detect antineuronal antibody staining in all patients with acute chorea, whereas healthy control subjects showed a complete absence of antineuronal antibodies.[38] Contrary to the previous studies,[16,21,22] which used fresh human brains from adult victims of sudden or accidental death, this study used a fresh human brain obtained from an autopsied stillborn cadaver, aged 34 weeks. It would be of interest to apply the same methodology in TS patients.

Measuring levels of antineuronal antibodies more quantitatively, by applying an enzyme-linked immunosorbent assay (ELISA) with human neural tissue from basal ganglia as substrate, TS subjects were shown to have a significant increase in the mean and median ELISA optical density (OD) levels of serum antibodies against putamen, but not against caudate or globus pallidus, when compared with healthy controls. Differences in magnitude of binding were, however, relatively low.[23] These results suggest that the putamen may be a major target site for antineuronal antibodies.

Two other studies did not use human neural tissue but an immortal cell line of neural origin as substrate, and also applied ELISA assays. Though using the same methodology, results were conflicting. When described originally, the assay was shown to have the capability to differentiate between normal and TS populations, with a sensitivity and specificity for TS of 79.1% and 61.2%, respectively.[24] A later study, however, did not find differences between children with TS and control children, due to high OD values in controls in this study.[25]

Finally, a recent study assessed the presence of antineuronal antibodies in subjects with TS and healthy controls with an indirect immunofluorescence assay on unfixed frozen sections from rat brain striatum, and performed serum titrations to an end

point by two-fold serial dilutions in order to quantify the levels of immunoreactivity.[26] Again, there was much overlap in levels of immunoreactivity between patients and controls. It is unclear, how to interpret this overlap between patients and controls. It raises suspicions of methodological flaws, suggesting rather high levels of nonspecific binding, albeit not necessarily, since autoantibodies in healthy subjects have repeatedly been reported.[39] Clearly, according to what criteria an individual's antineuronal antibody assay should be regarded positive has insufficiently been operationalized as yet.

Many questions remain regarding the significance, magnitude, and patho-physiological meaning of serum reactivity with neuronal tissue in tic disorders. In paraneoplastic syndromes (PNS) involving the central nervous system, the role of antineuronal antibodies is much more established. First, in this area, the nature of several neuronal antigens has been characterized. Furthermore, end-point titers for positive identification of antibody, in general, are much higher than is the case in movement disorders. Mostly, titers of > 1:500 will be regarded as positive in the field of PNS, whereas most titers do not exceed 1:8 when movement disorders are concerned.[40] Given these apparently low titers in tic disorders, discriminating differences from controls are fairly small. Correspondingly, confounds of nonspecific binding form a great risk when studying antineuronal antibody status in patients with tic disorders, paralleled by relatively high levels of positive reactivity in normal controls reported so far.

To conclude, though several studies suggest the presence of autoantibodies reacting with brain tissue in patients with OCD and/or tic disorders, some of these raise concerns regarding methodology. Moreover, the presence of autoantibodies in the serum of patients with tic disorders does not necessarily point to an autoimmune basis of tic disorder. Autoantibodies are also found when tissue damage is caused by trauma or infection. In other words, autoantibodies can result from, rather, than be the cause of tissue damage, as has been reviewed elsewhere.[41] One recent study, however, elegantly suggested a pathogenic role for antineuronal antibodies for tic disorders. Through the transfer of antineuronal antibodies of children with TS to the striatum of rats, stereotypic movements and utterances could be induced in these animals.[42]

Much remains to be investigated in this area, eg, how autoantibodies present in sera are able to cross the blood-brain barrier, or whether IgG production takes place inside the central nervous system. Simply measuring albumin and total IgG in serum as well as in cerebrospinal fluid would give valuable information about IgG production in the central nervous system and about the functional integrity of the blood-brain barrier. Recently, basic principles of these measurements have been adequately reviewed.[43] Theoretically, in this respect, it would be also of interest, to compare antineuronal antibodies instead of total IgG in both serum and cerobrospinal fluid. Contrary to total IgG, however, we lack accurate quantitative methods of measuring levels of antineuronal antibodies, as has been outlined in the previous sections.

Also, the way in which antibodies against neuronal antigens might induce tics and associated features is unknown. No evidence of antibody-associated inflammation or

indications of autoantibody binding to functional cell-surface receptors in central nervous tissue have been collected in the field of tic and related disorders. An animal model as described above,[42] or careful postmortem neuropathological examinations may be fruitful future approaches in this respect.

Association with antistreptococcal antibody titers

Conflicting results have also been reported on the issue whether increased levels of antistreptococcal antibodies (antistreptolysin O and antideoxyribunuclease B) are associated with TS. Two recent studies[27,28] reported large differences between TS cases and normal controls. Muller et al.[27] found that 85% of the subjects with TS versus 8% of normal controls had elevated antideoxyribunuclease B levels. The same researchers subsequently showed the presence in TS patients of increased titers against the streptococcal M12 and M19 proteins in TS patients as compared with controls, while antibody titers against M1, M4, and M6 did not differ between the TS and control groups.[29] Also, Cardona et al.[28] reported significantly higher mean antistreptolysin titers in children with tics compared to control children, and found a positive correlation between antistreptolysin titers and severity of tic disorder as measured by the Yale Global Tic Severity Scale. Others, however, reported less striking differences in this respect.[23]

Despite some inconsistency in these data, overall, a clear association is noticeable between antistreptococcal antibody titers and tic disorders, further strengthening the relationship between tic disorders and preceding streptococcal infections. Longitudinal data, however, intending to link symptom fluctuations over time to fluctuations of antistreptococcal antibody titers are highly needed. In addition, though Muller et al.[27] did not find increased antistreptococcal antibody titers in a comparison group of patients with schizophrenia, it remains to be systematically investigated whether elevated antistreptococcal antibody titers would also be associated with other related neuropsychiatric disorders such as OCD, autism spectrum disorders, and anorexia nervosa, or other mental disorders in which mechanisms of autoimmunity sometimes have been suggested, such as affective disorders.

Studies demonstrating elevated D8/17 expression on B lymphocytes, a marker of rheumatic fever

One finding which somehow links tic disorders to rheumatic fever is the greater than usual binding of a D8/17-specific monoclonal antibody to B lymphocytes, reported in patient groups of both disorders.[22] D8/17-specific monoclonal antibody is a mouse monoclonal IgM antibody originally prepared from fusions of

spleen cells from mice that had been repeatedly immunized with isolated human B cells obtained from patients with rheumatic fever or rheumatic heart disease. Elevated D8/17 B cell expression was originally investigated as a putative susceptibility marker of rheumatic fever.[44] Two independent research centers reported elevated D8/17 expression on B cells in patients with tic disorders. In these studies, B cells were incubated with the D8/17-specific monoclonal antibody and an anti-mouse IgM specific conjugate, after which D8/17-positive cells were counted by means of fluorescence microscopy.[22,30] Using the same, somewhat subjective method, elevated D8/17 B cell binding in autism has also been reported.[45] We recently demonstrated higher than usual D8/17 overexpression compared to a control monoclonal antibody in patients with a tic disorder, by means of flow cytometry, an objective rating method in which no operator variability is involved.[31] A significant minority of our patients (39.4%), however, had levels of D8/17 expression within the range of that of our healthy comparison subjects.

The exact meaning and pathogenetic significance of these findings is unknown. The D8/17-specific monoclonal antibody has not only been found to bind to B cell surface structures, but also to the cytoskeletal helical coil/coiled structures myosin and tropomyosin, as well as to streptococcal M proteins, possibly suggesting a substrate for structural homologies between host and streptococci.[46] Given the specificity of elevated D8/17 B cell expression for poststreptococcal disorders as reported after extensive studies with this antibody across different autoimmune disease categories,[47] elevated D8/17 B cell expression might point to the involvement of poststreptococcal (auto)immunity in tic disorders. Whether, however, the finding of elevated B cell expression in autistic subjects[45] also implies the involvement of poststreptococcal (auto)immunity in the pathophysiology of autistic disorder, remains to be specifically investigated. In other words, the precise meaning of elevated D8/17 expression remains obscure.

Altered tryptophan metabolism and data on cytokines

One well-known and sensitive marker of cellular immune activation is the increased degradation of tryptophan via the kynurenine pathway, leading to elevated plasma levels of kynurenine and subsequent metabolites.[48] Upregulation of the kynurenine pathway can be induced through increased activity of indoleamine 2,3-dioxygenase (IDO), an enzyme active in extra-hepatic tissue including brain, which is sensitive to interferon-gamma, a major cytokine of cellular immunity.[49]

Indeed, in one study, involving only seven patients with a tic disorder, the serum kynurenine level was found to be clearly increased in all seven patients, whereas serum tryptophan was normal,[32] thus, possibly reflecting immune activation. In a subsequent larger scale study,[33] involving 72 TS patients and 46 matched controls, again, plasma kynurenine levels were found to be significantly elevated. Interestingly, both studies[32,33] reported a significant positive correlation in TS patients between levels of

kynurenine and neopterin. This finding further supports involvement of (auto)immunity, given the fact that neopterin is a well-known marker of cellular immunity, which is, like IDO activity, induced by cytokines. We know of one other independent report of increased plasma kynurenine,[34] further strengthening the significance of this finding.

Also, significantly decreased serum tryptophan levels of TS patients have been reported in two large-scale studies,[50,51] which would be compatible with increased turnover of tryptophan along the kynurenine pathway. However, cerebrospinal fluid tryptophan levels were found to be normal in a different study.[52] Interestingly, conversion of tryptophan to kynurenine can be triggered by GABHS, as has been recently shown *in vitro*.[53] In that study, streptococcal erythrogenic toxins, exposed to a peripheral blood mononuclear cell culture were demonstrated to stimulate tryptophan degradation to kynurenine.

An alternative explanation for increased plasma kynurenine, other than through immune based IDO activation, would be activation of cortisol-inducible tryptophan dioxygenase (TDO), possibly reflecting heightened stress response.[54] However, contrary to neopterin, cortisol was not found to show a correlation with kynurenine.[33] Surprisingly, the studies reporting on elevated plasma kynurenine[32-34] did not yield elevated levels of quinolinic acid and kynurenic acid, two further metabolites along the kynurenine pathway.[55] Increased levels of quinolinic acid are known to be correlated with different immune-based neurologic conditions, among others the AIDS dementia complex.[56,57] When present in higher than usual levels in brain tissue, quinolinic acid is toxic to neurons, leading to a loss of neuronal cell density. Cerebrospinal fluid levels of quinolinic acid are closely associated with severity of neurologic damage in inflammatory brain disease.[56,58] Therefore, it would be of interest, to study kynurenine pathway metabolites in cerebrospinal fluid of TS patients.

Precisely how an altered metabolism of tryptophan might contribute to the pathogenesis of tics is not clear. It can be an epiphenomenon, merely reflecting a state of immune activation. Another possibility is a direct toxic effect of kynurenine or one of its metabolites in the basal ganglia. Some authors found that kynurenine increases tic-like behavior in an animal model of TS: in mice, head-shakes which had been induced by the 5-hydroxytryptamine receptor agonist dimethoxy-iodophenyl-aminopropane were potentiated by administration of kynurenine.[59] A third possibility would be that reduced levels of tryptophan could lead to reduced synthesis of serotonin, causing pertuberations in serotonergic transmission. The last two possibilities are not mutually exclusive.

Clearly, however, more data are needed concerning altered tryptophan metabolism in patients with tic and related disorders, to allow for more definite conclusions. Unfortunately, studies which investigated cytokines in pediatric neuropsychiatric disease are scarce. Patients with OCD were reported in one study to show a relative preponderance in cerebrospinal fluid of type 1 cytokines, notably interleukine-2, suggesting the involvement of cell-mediated immunity.[35] This could be consistent with a role for streptococcal infection, through the involvement of streptococcal erythrogenic toxins, which can act as superantigens and are known to induce type 1 cytokines.[53,60]

Cytokines are proteins made by cells that affect the behavior of other cells. One function of cytokines is to shape the type of adaptive immunity in response to a pathogen, by determining the fate of naive CD4 T cells. Proliferating CD4 T cells can differentiate into type 1 CD4 T cells (Th1 cells) or type 2 CD4 T cells (Th2 cells), which mainly depends on the type of cytokines produced in response to pathogens by cells of the innate nonadaptive immune system. Th1 cells are involved in activating macrophages, resulting in cell-mediated immunity, whereas Th2 cells have their main function in stimulating B cells to become antibody producing plasma cells.

Given the known IDO induction capacities of interferon-gamma, it would be of interest to study cytokine profiles in patients with tic disorders. At present, no such studies are available.

Application of immunomodulatory interventions in tic disorders

One attractive consequence of the accumulating evidence supporting the involvement of autoimmunity in the pathophysiology of tic disorders is the development of new treatment options, targeting on the supposed pathophysiology instead of being purely symptomatic. The existing literature concerning immunomodulatory therapy for TS and related disorders is still surprisingly scarce, and is confined to a handful of case studies and one placebo-controlled study.

A small number of case studies reported improvement in tic severity after immunosuppression with corticosteroids.[61-63] Other case studies in the literature reported dramatic symptom improvement in children fulfilling criteria for PANDAS with either plasmapheresis,[18-20] or intravenous immunoglobulin (IVIG).[20,64,65] In some of these case studies, after succesful plasmapheresis, attempts have been made to prevent new GABHS infections with penicillin prophylaxis.[20]

We know of only one placebo-controlled study on the efficacy of immunomodulatory therapy in patients with TS/OCD.[36] In that study, a total number of 30 children meeting PANDAS criteria, were randomly assigned to either plasmapheresis, IVIG, or a placebo condition (saline solution given in the same manner as IVIG), and subsequently each patient was given a regimen of penicillin prophylaxis. Symptom severity was rated at baseline and at 1 month and 12 months after treatment by use of standard assessment scales for OCD, tics, anxiety, depression, and global functioning. At 1 month after treatment the IVIG/placebo masking was broken. Then, the IVIG and plasma exchange groups showed striking improvements in severity of OCD symptoms, anxiety, and overall functioning. Tic symptoms were significantly improved by plasma exchange only. The children in the placebo condition did not show any amelioration. Interestingly, these improvements were maintained at 1 year after treatment for both plasmapheresis and IVIG. Though these results are intriguing, the study of Perlmutter and colleagues has some serious drawbacks. At baseline, tic severity in the plasma exchange group was significantly higher than in the IVIG and sham-IVIG groups, which makes it hard to evaluate the

effect of IVIG on tic severity. Blinding of the treatment groups was already broken at 1 month after treatment. In fact, the study lacks an acceptable sustained placebo condition. Another serious drawback is the limited number of symptom rating moments, both before and after treatment. As a consequence, we lack knowledge of the way of natural symptom fluctuation in the groups versus the possible effect of intervention.

Perlmutter and colleagues did not use laboratory measures in their assessment after treatment, so from Perlmutter's data, we cannot gain insight in possible treatment mechanisms. More specifically, baseline and post-treatment levels of antineuronal antibodies and percentages of D8/17-positive B lymphocytes would have been of interest. Two more problems are the small number of patients in each treatment arm and the confounding role of the use of penicillin.

Certainly, further, larger scale studies on the effectiveness of immune-based therapies in well-characterized patients with tic disorders would be of great interest.

How valuable is the PANDAS concept?

Many problems are associated with the concept of PANDAS, both methodologically and fundamentally. The diagnostic criteria for PANDAS are not easy to apply. Especially, it is hard to demonstrate a temporal association between GABHS infection and symptom onset or exacerbations. In fact, as far as such an association in tic disorder or OCD exists, no knowledge is available of the nature of this association. Interestingly, in Sydenham's chorea, a latent period between onset of symptoms and the preceding GABHS infection as long as 6 months is not uncommon.[66] Streptococcal infections are fairly common in children in general,[67] as are remissions and exacerbations in children with tic disorders.[68] Another criterion for PANDAS, the presence of an episodic course of symptom severity, with explosive exacerbations and remissions, has insufficiently been operationalized. An episodic course of symptoms is characteristic of pediatric tic and OCD symptoms in general, but it is hard to say when exacerbations can exactly be called explosive.

The significance and validity of PANDAS remains to be established, since we lack direct comparisons between TS subjects who do, and TS subjects who do not meet criteria for PANDAS. Comparative studies in this respect should include comprehensive clinical and serological features in relation to response to treatment. Besides, much of the evidence supporting a role for autoimmunity in tic disorders in general has been based on unselected subjects. Findings in unselected cases have been similar in magnitude as in cases fulfilling criteria for PANDAS. All of the findings supporting autoimmunity as outlined in the previous sections are equally valid for unselected TS patients. These include the D8/17 B cell overexpression[22,31] and levels of increased antineuronal autoantibodies,[24] both of which are the most robust indicators of a role for autoimmunity. There is, however, one major exception: researchers at the NIMH recently conducted an open trial of plasma exchange in five patients with non-PANDAS OCD, after which none showed

significant improvement.[69] To fully investigate the usefulness of the proposed PANDAS concept, more studies comparing TS subjects fulfilling the PANDAS criteria with non-PANDAS subjects will have to be performed. At present, it suffices to state that PANDAS probably simply represents those patients who feature the most obvious relationship with GABHS infections.

Apart from the point whether or not PANDAS is a useful concept, one may question, what type of symptoms should be included in the spectrum of tic and related disorders. The PANDAS criteria simply require the presence of OCD and/or a tic disorder as the first criterion,[70] thus, lumping together two types of disorders which are generally regarded distinct in phenomenology and presumed etiology.[71] Only a subgroup of pediatric OCD may be etiologically related to tic disorders, characterized by a family history of tics, a more familial subtype in general, male preponderance, association with disruptive behavior and developmental disorders, and a less striking association with mood disorders.[72] The predominant phenomenology of obsessions/compulsions also differs between tic-related and non-tic-related OCD. Presence of hoarding/symmetry and sexual and aggressive symptoms predominates in the former, whereas in non-tic-related OCD, symptoms of contamination/checking prevail.[73] In tic-related OCD, compulsions do not generally appear to be anxiety-driven, but rather, could be phenomenologically regarded as complex tic behaviors. It is well-known that so-called mental tics form a part of tic symptomatology, and it is our clinical impression that in many cases, certain obsessions and compulsions could be better conceptualized as mental and complex tics, respectively. Thus, it may well be that the presence of tics, or, more general, a movement disorder, in contrast to anxiety-driven obsessions and compulsions, forms the distinctive feature of those neuropsychiatric disorders which may be related to autoimmunity. Our preliminary, unpublished data on D8/17 B cell overexpression in OCD suggest, that only tic-related OCD shows increased expression of this marker of rheumatic fever.

In conclusion, the concept of PANDAS is ill-defined, is not supported by unique immunologic findings which have not been reported in unselected patients, and is phenomenologically unsound. It would be better to define homogeneous subgroups by means of both clinical and laboratory characteristics. Presence of antineuronal antibodies, or levels of D8/17 B cell overexpression might be two candidate approaches.

Conclusions and future directions

A growing body of research data indicates the involvement of autoimmunity in the pathogenesis of at least a subgroup of patients with tic and related disorders. The most fascinating data include the work on antineuronal autoantibodies,[21-26] especially regarding their potential in generating disease in an animal model,[42] the association with B lymphocyte D8/17 expression,[22,30,31] and the promising data concerning immunomodulatory approaches[36] in a disorder which is otherwise hard to manage. Given the economic costs and the invasiveness of these interventions,

future research should focus on the identification of patients in whom autoimmunity may be involved, and who may subsequently profit from immunomodulatory treatments.

Much more work remains to be done in this field, however. The characterization of the antigen recognized by the D8/17-specific monoclonal antibody as well as the characterization of the brain antigenic structures recognized by the antineuronal antibodies awaits further study. Western blot analyses which can identify antibodies against specific antigenic structures have been inconclusive so far.[23] Postmortem studies in search of inflammatory alterations warrant further attention.

Furthermore, large-scale longitudinal data are needed to investigate the role of infections with regard to symptom fluctuation. Many areas remain neglected. Just a few studies examined the human leukocyte antigen (HLA) system.[74-76] Since many human autoimmune diseases show HLA-linked disease associations, studying HLA-associations would be of great interest in homogeneous, immune-based subgroups of tic disorders. Also, we lack imaging studies aimed at visualizing possible blood-brain barrier ruptures. Of special interest in this regard is the report by Kienzle and coworkers[77] demonstrating focal blood-brain barrier disruption confined to the head of the caudate nucleus in two patients with Sydenham's chorea during the active phase of the disease, by magnetic resonance imaging after intravenous administration of gadopentetate dimeglumine. Interestingly, when repeating the procedure in these patients after symptoms had greatly diminished, leakage of contrast was absent.

Ultimately, unraveling the genetic background of tic and related disorders will hopefully lead to a better understanding of environmental factors and more targeted treatment.

References

1. Peterson BS, Leckman JF. The temporal dynamics of tics in Gilles de la Tourette syndrome. *Biol Psychiatry* 1998;44:1337-48.
2. Coffey BJ, Miguel EC, Savage CR, Rauch SL. Tourette's disorder and related problems: a review and update. *Harv Rev Psychiatry* 1994;2:121-32.
3. Bruun RD, Budman CL. The course and prognosis of Tourette syndrome. *Neurol Clin* 1997;15:291-8.
4. Kurlan R, Behr J, Medved L. Transient tic disorder and the clinical spectrum of Tourette's syndrome. *Arch Neurol* 1988;45:1200-1.
5. Tanner CM, Goldman SM. Epidemiology of Tourette syndrome. *Neurol Clin* 1997;15:395-402.
6. Jankovic J. Tourette syndrome. Phenomenology and classification of tics. *Neurol Clin* 1997;15:267-75.
7. Coffey BJ, Park KS. Behavioral and emotional aspects of Tourette syndrome. *Neurol Clin* 1997;15:277-89.
8. Knell ER, Comings DE. Tourette's syndrome and attention-deficit hyperactivity disorder: evidence for a genetic relationship. *J Clin Psychiatry* 1993;54:331-7.
9. Muller N, Putz A, Kathmann N, Lehle R, Gunther W, Straube A. Characteristics of obsessive-compulsive symptoms in Tourette's syndrome, obsessive-compulsive disorder, and Parkinson's disease. *Psychiatry Res* 1997;70:105-14.
10. Kurlan R, Daragjati C, Como PG, McDermott MP, Trinidad KS, Roddy S, *et al.* Non-obscene complex socially inappropriate behavior in Tourette's syndrome. *J Neuropsychiatry Clin Neurosci* 1996;8:311-7.
11. Leckman JF, Peterson BS, Anderson GM, Arnsten AF, Pauls DL, Cohen DJ. Pathogenesis of Tourette's syndrome. *J Child Psychol Psychiatry* 1997;38:119-42.
12. Alsobrook JP, Pauls DL. The genetics of Tourette syndrome. *Neurol Clin* 1997;15:381-93.
13. Barr CL, Wigg KG, Pakstis AJ, Kurlan R, Pauls D, Kidd KK, *et al.* Genome scan for linkage to Gilles de la Tourette syndrome. *Am J Med Genet* 1999;88:437-45.
14. Kurlan R. Tourette syndrome. Treatment of tics. *Neurol Clin* 1997;15:403-9.
15. Swedo SE. Sydenham's chorea. A model for childhood autoimmune neuropsychiatric disorders. *JAMA* 1994;272:1788-91.
16. Swedo SE, Leonard HL, Schapiro MB, Casey BJ, Mannheim GB, Lenane MC, *et al*. Sydenham's chorea: physical and psychological symptoms of St Vitus dance. *Pediatrics* 1993;91:706-13.
17. Steingard R, Dillon-Stout D. Tourette's syndrome and obsessive compulsive disorder. Clinical aspects. *Psychiatr Clin North Am* 1992;15:849-60.
18. Tucker DM, Leckman JF, Scahill L, Wilf GE, LaCamera R, Cardona L, *et al*. A putative poststreptococcal case of OCD with chronic tic disorder, not otherwise specified. *J Am Acad Child Adolesc Psychiatry* 1996;35:1684-91.
19. Giedd JN, Rapoport JL, Leonard HL, Richter D, Swedo SE. Case study: acute basal ganglia enlargement and obsessive-compulsive symptoms in an adolescent boy. *J Am Acad Child Adolesc Psychiatry* 1996;35:913-5.
20. Allen AJ, Leonard HL, Swedo SE. Case study: a new infection-triggered, autoimmune subtype of pediatric OCD and Tourette's syndrome. *J Am Acad Child Adolesc Psychiatry* 1995;34:307-11.
21. Kiessling LS, Marcotte AC, Culpepper L. Antineuronal antibodies in movement disorders. *Pediatrics* 1993;92:39-43.
22. Murphy TK, Goodman WK, Fudge MW, Williams RC, Jr., Ayoub EM, Dalal M, *et al*. B lymphocyte antigen D8/17: a peripheral marker for childhood-onset obsessive-compulsive disorder and Tourette's syndrome? *Am J Psychiatry* 1997;154:402-7.
23. Singer HS, Giuliano JD, Hansen BH, Hallett JJ, Laurino JP, Benson M, *et al*. Antibodies against human putamen in children with Tourette syndrome. *Neurology* 1998;50:1618-24.
24. Laurino JP, Hallett J, Kiessling LS, Benson M, Pelletier T, Kuhn C. An

immunoassay for anti-neuronal antibodies associated with involuntary repetitive movement disorders. *Ann Clin Lab Sci* 1997;27:230-5.

25. Singer HS, Giuliano JD, Hansen BH, Hallett JJ, Laurino JP, Benson M, *et al*. Antibodies against a neuron-like (HTB-10 neuroblastoma) cell in children with Tourette syndrome. *Biol Psychiatry* 1999;46:775-80.

26. Morshed SA, Parveen S, Leckman JF, Mercadante MT, Bittencourt Kiss MH, Miguel EC, *et al.* Antibodies against Neural, Nuclear, Cytoskeletal and Streptococcal Epitopes in Children and Adlts with Tourette's Syndrome, Sydenham's Chorea and Autoimmune Disorders. *Submitted for publication.*

27. Muller N, Riedel M, Straube A, Gunther W, Wilske B. Increased anti-streptococcal antibodies in patients with Tourette's syndrome. *Psychiatry Res* 2000;94:43-9.

28. Cardona F, Orefici G. Group A streptococcal infections and tic disorders in an Italian pediatric population. *J Pediatr* 2001;138:71-5.

29. Muller N, Kroll B, Schwarz MJ, Riedel M, Straube A, Lutticken R, *et al.* Increased titers of antibodies against streptococcal M12 and M19 proteins in patients with Tourette's syndrome. *Psychiatry Res* 2001;25:187-193.

30. Swedo SE, Leonard HL, Mittleman BB, Allen AJ, Rapoport JL, Dow SP, *et al*. Identification of children with pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections by a marker associated with rheumatic fever. *Am J Psychiatry* 1997;154:110-2.

31. Hoekstra PJ, Bijzet J, Limburg PC, Steenhuis MP, Troost PW, Oosterhoff MD, *et al*. Elevated D8/17 expression on B-Lymphocytes, a marker associated with rheumatic fever, in tic disorder patients, measured with flow cytometry. *Am J Psychiatry* 2001;158: 605-10.

32. Rickards H, Dursun SM, Farrar G, Betts T, Corbett JA, *et al.* Increased plasma kynurenine and its relationship to neopterin and tryptophan in Tourette's syndrome. *Psychol Med* 1996;26:857-62.

33. Gaynor CM, Rickards EHG, Kariyawasam SH, Sidey FM, Robertson MM, Corbett JA, *et al* Increased plasma kynurenine in Tourette syndrome may be due to induction of IDO [Abstract]. *J Psychopharmacol Suppl* 1997;11:A23.

34. Chappel PB, Anderson GM, Goodman WK, Price LH, Hall LM, Cohen DJ, *et al.* Kynurenine pathway metabolites in CSF and plasma of Tourette syndrome patients [Abstract]. *Abstr Soc Neurosci* 1995;21:1111.

35. Mittleman BB, Castellanos FX, Jacobsen LK, Rapoport JL, Swedo SE, Shearer GM. Cerebrospinal fluid cytokines in pediatric neuropsychiatric disease. *J Immunol* 1997;159:2994-9.

36. Perlmutter SJ, Leitman SF, Garvey MA, Hamburger S, Feldman E, Leonard HL, *et al*. Therapeutic plasma exchange and intravenous immunoglobulin for obsessive-compulsive disorder and tic disorders in childhood. *Lancet* 1999;354:1153-8.

37. Husby G, van de Rijn I, Zabriskie JB, Abdin ZH, Williams RC, Jr. Antibodies reacting with cytoplasm of subthalamic and caudate nuclei neurons in chorea and acute rheumatic fever. *J Exp Med* 1976;144:1094-110.

38. Kotby AA, El Badawy N, El Sokkary S, Moawad H, El Shawarby M. Antineuronal antibodies in rheumatic chorea. *Clin Diagn Lab Immunol* 1998;5:836-9.

39. Lacroix-Desmazes S, Kaveri SV, Mouthon L, Ayouba A, Malanchere E, Coutinho A, *et al.* Self-reactive antibodies (natural autoantibodies) in healthy individuals. *J Immunol Methods* 1998;216:117-37.

40. Moll JW, Antoine JC, Brashear HR, Delattre J, Drlicek M, Dropcho EJ, *et al.* Guidelines on the detection of paraneoplastic anti-neuronal-specific antibodies: report from the Workshop to the Fourth Meeting of the International Society of Neuro-Immunology on paraneoplastic neurological disease, held October 22-23, 1994, in Rotterdam, The Netherlands. *Neurology* 1995;45:1937-41.

41. Naparstek Y, Plotz PH. The role of autoantibodies in autoimmune disease. *Annu Rev Immunol* 1993;11:79-104.

42. Hallett JJ, Harling-Berg CJ, Knopf PM, Stopa EG, Kiessling LS. Anti-striatal antibodies in tourette syndrome cause neuronal dysfunction. *J Neuroimmunol* 2000;111:195-202.

43. Reiber H, Peter JB. Cerebrospinal fluid analysis: disease-related data patterns and evaluation programs. *J Neurol Sci* 2001;184:101-22.

44. Khanna AK, Buskirk DR, Williams RC, Jr., Gibofsky A, Crow MK, Menon A, *et al.* Presence of a non-HLA B cell antigen in rheumatic fever patients and their families as defined by a monoclonal antibody. *J Clin Invest* 1989;83:1710-6.

45. Hollander E, Delgiudice-Asch G, Simon L, Schmeidler J, Cartwright C, Decaria CM, *et al*. B lymphocyte antigen D8/17 and repetitive behaviors in autism. *Am J Psychiatry* 1999;156:317-20.

46. Kemeny E, Husby G, Williams RC, Jr., Zabriskie JB. Tissue distribution of antigen(s) defined by monoclonal antibody D8/17 reacting with B lymphocytes of patients with rheumatic heart disease. *Clin Immunol Immunopathol* 1994;72:35-43.

47. Gibofsky A, Khanna A, Suh E, Zabriskie JB. The genetics of rheumatic fever: relationship to streptococcal infection and autoimmune disease. *J Rheumatol Suppl* 1991;30:1-5.

48. Heyes MP.The kynurenine pathway and neurologic disease. Therapeutic strategies. *Adv Exp Med Biol* 1996*;*398:125-9

49. Meyer KC, Arend RA, Kalayoglu MV, Rosenthal NS, Byrne GI, Brown RR. Tryptophan metabolism in chronic inflammatory lung disease. *J Lab Clin Med* 1995;126:530-40.

50. Comings DE. Blood serotonin and tryptophan in Tourette syndrome. *Am J Med Genet* 1990;36:418-30.

51. Leckman JF, Anderson GM, Cohen DJ, Ort S, Harcherik DF, Hoder EL, *et al.* Whole blood serotonin and tryptophan levels in Tourette's disorder: effects of acute and chronic clonidine treatment. *Life Sci* 1984;35:2497-503.

52. Leckman JF, Goodman WK, Anderson GM, Riddle MA, Chappell PB, McSwiggan-Hardin MT, *et al*. Cerebrospinal fluid biogenic amines in obsessive compulsive disorder, Tourette's syndrome, and healthy controls. *Neuropsychopharmacology* 1995;12:73-86.

53. Murr C, Widner B, Gerlach D, Werner-Felmayer G, Dierich MP, Wachter H, *et al.* Streptococcal erythrogenic toxins induce tryptophan degradation in human peripheral blood mononuclear cells. *Int Arch Allergy Immunol* 1997;114:224-8.

54. Danesch U, Gloss B, Schmid W, Schutz G, Schule R, Renkawitz R. Glucocorticoid induction of the rat tryptophan oxygenase gene is mediated by two widely separated glucocorticoid-responsive elements. *EMBO J* 1987;6:625-30.

55. Heyes MP, Chen CY, Major EO, Saito K. Different kynurenine pathway enzymes limit quinolinic acid formation by various human cell types. *Biochem J* 1997;326 (Pt 2):351-6.

56. Sardar AM, Bell JE, Reynolds GP. Increased concentrations of the neurotoxin 3-hydroxykynurenine in the frontal cortex of HIV-1-positive patients. *J Neurochem* 1995;64:932-5.

57. Heyes MP, Jordan EK, Lee K, Saito K, Frank JA, Snoy PJ, *et al.* Relationship of neurologic status in macaques infected with the simian immunodeficiency virus to cerebrospinal fluid quinolinic acid and kynurenic acid. *Brain Res* 1992;570:237-50.

58. Heyes MP, Saito K, Crowley JS, Davis LE, Demitrack MA, Der M, *et al.* Quinolinic acid and kynurenine pathway metabolism in inflammatory and non-inflammatory neurological disease. *Brain* 1992;115 (Pt 5):1249-73.

59. McCreary AC, Handley SL. Kynurenine potentiates the DOI headshake in mice. *J Psychopharmacology* 1995;9:68-69.

60. Bhatnagar A, Grover A, Ganguly NK. Superantigen-induced T cell responses in acute rheumatic fever and chronic rheumatic heart disease patients. *Clin Exp Immunol* 1999;116:100-6.

61. Geschwind N, Kondo K. Corticosteroid therapy and Tourette syndrome [letter]. *Ann Neurol* 1979;5:495.

62. Kondo K, Kabasawa T. Improvement in Gilles de la Tourette syndrome after corticosteroid therapy. *Ann Neurol* 1978;4:387.

63. Matarazzo EB. Tourette's syndrome treated with ACTH and prednisone: Report of two cases. *Journal Child Adolesc Psychopharmacol* 1992;2:215-26.

64. Muller N, Riedel M, Erfurth A, Moller HJ. Immunoglobulin therapy in Gilles de la Tourette syndrome. *Nervenarzt* 1997;68:914-6.

65. Perlmutter SJ, Garvey MA, Castellanos X, Mittleman BB, Giedd J, Rapoport JL, *et al*. A case of pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections. *Am J Psychiatry* 1998;155:1592-8.

66. Marques-Dias MJ, Mercadante MT, Tucker D, Lombroso P. Sydenham's chorea. *Psychiatr Clin North Am* 1997;20:809-20.

67. De Wals P, Bouckaert A. Methods for estimating the duration of bacterial carriage. *Int J Epidemiol* 1985;14:628-34.

68. Singer HS, Giuliano JD, Zimmerman AM, Walkup JT. Infection: a stimulus for tic disorders. *Pediatr Neurol* 2000;22:380-3.

69. Nicolson R, Swedo SE, Lenane M, Bedwell J, Wudarsky M, Gochman P, *et al*. An open trial of plasma exchange in childhood-onset obsessive-compulsive disorder without poststreptococcal exacerbations. *J Am Acad Child Adolesc Psychiatry* 2000;39:1313-5.

70. Garvey MA, Giedd J, Swedo SE. PANDAS: the search for environmental triggers of pediatric neuropsychiatric disorders. Lessons from rheumatic fever. *J Child Neurol* 1998;13:413-23.

71. Cavallini MC, Pasquale L, Bellodi L, Smeraldi E. Complex segregation analysis for obsessive compulsive disorder and related disorders. *Am J Med Genet* 1999;88:38-43.

72. Leonard HL, Swedo SE, Garvey M, Beer D, Perlmutter S, Lougee L, *et al*. Postinfectious and other forms of obsessive-compulsive disorder. *Child Adolesc Psychiatr Clin N Am* 1999;8:497-511.

73. Petter T, Richter MA, Sandor P. Clinical features distinguishing patients with Tourette's syndrome and obsessive-compulsive disorder from patients with obsessive-compulsive disorder without tics. *J Clin Psychiatry* 1998;59:456-9.

74. Min SK, Lee H, Park KI, Park MS, Namkoong K. Tourette disorder and HLA typing. *Yonsei Med J* 1991;32:315-8.

75. Caine ED, Weitkamp LR, Chiverton P, Guttormsen S, Yagnow R, Hempfling S, *et al*. Tourette syndrome and HLA. *J Neurol Sci* 1985;69:201-6.

76. Comings DE, Gursey BT, Hecht T, Blume K. HLA typing in Tourette syndrome. *Adv Neurol* 1982;35:251-3.

77. Kienzle GD, Breger RK, Chun RW, Zupanc ML, Sackett JF. Sydenham chorea: MR manifestations in two cases. *AJNR Am J Neuroradiol* 1991;12:73-6.

Chapter 2

Relative contribution of ADHD, OCD, and tic severity to social and behavior problems in tic disorders

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Abstract

Objective: *To investigate social and behavior problems related to attention deficit/hyperactivity disorder (ADHD), severity of obsessions and compulsions, and tic severity in children with a tic disorder.*

Methods: *Parents of 58 clinically referred children with a tic disorder with and without different forms of ADHD filled out the Child Behavior Checklist (CBCL) and the Children's Social Behavior Questionnaire, a novel parent questionnaire covering a broad range of behavior problems as seen in children with milder variants of pervasive developmental disorders. Comparisons were made between tic disorder patients with primarily hyperactive-impulsive ADHD, tic disorder patients with primarily inattentive ADHD, and tic disorder patients without ADHD. Also, part correlations of subscale scores of the questionnaires with measures of ADHD severity, severity of obsessions and compulsions, and tic severity, respectively, controlling for the predictive value of the other two measures on the outcome of the questionnaires were executed.*

Results: *Tic disorder patients with primarily hyperactive-impulsive ADHD had the highest scores on the parent questionnaires, tic disorder patients with primarily inattentive ADHD medium scores, and tic disorder patients without ADHD the lowest. On a considerable number of subscales, significant part correlations with ADHD severity, but not tic severity were obtained. Severity of obsessions and compulsions was independently correlated with the CBCL subscale thought problems, but not with most other subscales. There was no significant correlation between tic severity and ADHD severity.*

Conclusion: *In patients with a tic disorder, it is the presence and severity of ADHD that is the main predictor of associated behavior and social problems.*

Introduction

Though solely defined by the presence of recurrent motor movements and vocalizations, Tourette's disorder (TD) and other tic disorders are well-known to be associated with a wide range of psychopathology.[1] Often, behavioral abnormalities and problems in social and emotional adjustment of patients with a tic disorder are far more disabling than the core tic symptoms, thus, constituting a major challenge to treatment. With regard to the association of tic disorders with single (DSM-IV based) categorical entities of mental disorders, the available studies show considerable agreement. Many referred children have behavioral features of inattention, hyperactivity, and impulsivity, severe enough to justify a separate classification of attention deficit/hyperactivity disorder (ADHD).[2] The frequent presence of obsessive-compulsive disorder (OCD) in patients with a tic disorder is also apparent across different centers.[3-5] Some researchers furthermore reported on an association with mood and anxiety disorders.[6,7]

Another clinically well-known feature of many referred patients with a tic disorder constitutes their marked problems in social interaction, an associated feature that cannot easily be captured in DSM-IV based categories. There is a relative lack of studies specifically investigating problems in social functioning of patients with tic disorders. Champion et al.[8] reported more than 40% of patients with TD to have problems in dating and making and keeping friends. Stokes and colleagues[9] used the Pupil Evaluation Inventory, a sociometric questionnaire completed by the child's classmates, and found TD patients to be significantly more withdrawn, more aggressive, and less popular than their classmates. Another study[10] described the frequent occurrence of socially inappropriate behavior in patients with TD, such as insulting and inappropriately commenting on others. Singer and Rosenberg,[11] using the parent form of the Child Behavior Checklist (CBCL)[12] identified problems with obsessive-compulsive behavior, aggressiveness, hyperactivity, immaturity, withdrawal, and somatic complains in patients with TD. In that study, tic severity was not a statistically significant predictor of behavioral disturbance. A subsequent study,[13] also using the CBCL, did find a relationship between the severity of tics and behavioral problems.

The influence of co-occurring ADHD, however, is not well-corrected for in these studies. Social disability is a well-known phenomenon in patients with ADHD.[14] Given the frequent co-occurrence of ADHD and tic disorders, it is essential to study social behavior independently from the presence of ADHD, in order to disentangle the impact of ADHD. In one study that specified ADHD-status of children with TD, measures of peer relationships were not found to be related to tic severity. Instead, the co-occurrence of ADHD did turn out to be an important indicator of poor peer relationships in that study.[15] In another study,[16] using the CBCL and the Vineland Adaptive Behavior Scales, children with TD without ADHD were not significantly different on most measures of externalizing behaviors and social adaptation, as opposed to children with both TD and ADHD. The latter were characterized by significant behavior problems and poor social

adaptation. Thus, existing studies suggest that much of the social and behavioral maladaptation reported in earlier studies in children with TD is ADHD-specific.

The purpose of the present study was to investigate the role of ADHD versus tic and OCD severity in social and behavior problems of children with a tic disorder. We determined the association of subtypes of ADHD, as well as the number of ADHD symptoms compared to tic severity and severity of obsessions and compulsions, with social and behavioral functioning of patients with a tic disorder, by using the CBCL as well as the Children's Social Behavior Questionnaire (CSBQ). First, we made comparisons on these parent rating scales between tic disorder patients with primarily hyperactive-impulsive ADHD, tic disorder patients with primarily inattentive ADHD, and tic disorder patients without ADHD. In a second analysis, we measured the unique relative contribution of severity of tics, severity of obsessions and compulsions, and the number of ADHD symptoms to the behavioral and social problems of patients with a tic disorder.

The CSBQ is a novel parent questionnaire, containing items referring to behavior problems as seen in children with milder variants of pervasive developmental disorders and covering social interaction, communication, stereotyped behaviors, motor behavior, attention, affect regulation, sensory abnormalities, and children's understanding of social cues.[17] Previous research demonstrated the good psychometric qualities of the CSBQ with respect to both reliability and validity.[18] Also, the CSBQ has been shown to be a sensitive instrument to assess social problems in children with developmental problems including ADHD.[19]

Based on previous studies, we hypothesize that it is not the severity of tics, but the presence of ADHD and the number of ADHD symptoms that constitutes the major factor predicting social behavior problems as measured by the CBCL and the CSBQ.

Methods

Sample selection

Patients were recruited from two sources, either from members of the TD patients' association in the Netherlands or from patients who had previously (from 1989 through 1999) been referred to the outpatient clinic of the Child and Adolescent Psychiatry Center in Groningen, the Netherlands, and who had subsequently received a clinical diagnosis of a tic disorder. Parents of both groups received a written invitation to participate in the study. This letter fully described the aims and background of the study. Unfortunately, it cannot be said what percentage of subjects of both sources were willing to participate in the study. First, we did not a priori know how many members of the TD patients' association were between 4 and 18 years of age (the association has many adult members), so we do not know how many subjects in the desired age range we actually did approach. In addition, we were not sure how many addresses of former patients from our outpatient clinic were still correct, when we tried to contact them. Finally, some parents will have

received two letters, as far as they were both known to our clinic and the patients' association. However, to give an impression of response rates, of 461 letters sent to parents of patients under the age of 18 with a clinical diagnosis of a tic disorder from our outpatient clinic, 146 (or 32%) were willing to participate. In addition, 22 parents of subjects younger than 18 from the patients' association showed their willingness. Those parents who wanted to participate in the present study, were subsequently mailed a booklet containing parent questionnaires which had to be filled out prior to a scheduled meeting of the patient and their parents with one of the clinical investigators of the study, all experienced clinicians in the field of tic disorders.

Only patients between 4 and 18 years old who met Tourette's Syndrome Research Criteria for a definite tic disorder entered the study. According to these last criteria, observable tics had to be present during the clinical interview to allow for study entry. Of all 168 patients who originally were willing to participate and who showed up on the clinical interview, 58 were judged to have a definite tic disorder, and, thus, entered the study (five from the patients' association and 53 from our outpatient clinic). The remaining 110 patients either did not unequivocally demonstrate tics during the clinical interview, or had overgrown their tics. Forty-three of the participating patients met DSM-IV criteria for TD, the remaining 15 had a DSM-IV classification of chronic motor tic disorder. All five patients from the TD patients' association had in the past been referred to a mental health service. Thus, our final sample exclusively consisted of clinically referred patients. The only exclusion criteria were an existing clinical diagnosis of autistic disorder or a known IQ below 70. None of the subjects who were willing to participate in the study fulfilled one or both of these exclusion criteria.

Subject characteristics

A total number of 58 patients with a definite tic disorder participated (43 male, 15 female), ranging in age from 5 to 16 years (mean=11.4, SD=2.6). To assess the tic severity of the patients, we used the Yale Global Tic Severity Scale (YGTSS), consisting of separate scales for motor and vocal tics complemented with a separate rating of impairment. The mean total YGTSS score for the patients was 35.7 (SD=20.6, median=34.5, range=5-81), with a mean motor score of 11.7 (SD=4.0, median=11.0, range=4-21), a mean vocal score of 7.3 (SD=5.7, median=8.0, range=0-22), and a mean impairment score of 16.7 (SD=15.2, median=20.0, range=0-50). Since the impairment score of the YGTSS focuses on the impact of the tic disorder on the individual and does not measure tic severity per se, we decided to use the sum of the motor and vocal tic score as measure of objective tic severity. Mean objective tic severity for the patients was 19.0 (SD=6.6, median=18.0, range=5-41).

The children's Yale-Brown Obsessive Compulsive Scale (CYBOCS)[20] was used to measure severity of obsessions and compulsions. The mean obsessive score of the patients was 2.0 (SD=3.8, median=0.0, range=0-13), whereas the mean compulsive score was 3.0 (SD=4.8, median=0.0, range=0-16). Mean total CYBOCS score was 5.0 (SD=7.6, median=0.0, range=0-26).

Thirty-eight of the patients were using psychotropic medication, either various antipsychotic agents (N=17), clonidine (N=7), psychostimulants (N=3), a selective serotonin reuptake inhibitor (N=1), combinations of antipsychotic agents and clonidine (N=6), combinations of antipsychotic agents and psychostimulants (N=2), or combinations of antipsychotic agents and a selective serotonin reuptake inhibitor (N=2); 20 patients were free of medication.

To determine ADHD-status of the patients with a definite tic disorder, we made use of the ADHD part of the parent version of the Dutch translation of the Diagnostic Interview Schedule (DISC-P), which we administered in the absence of the child. This DSM-IV based semi-structured interview assesses whether or not a child fulfils each separate DSM-IV criterion for ADHD, thus resulting in a diagnosis of no ADHD, ADHD combined type, ADHD predominantly inattentive type, or ADHD predominantly hyperactive-impulsive type. The DISC-P algorithm only allows for a classification of an ADHD subtype, if significant symptoms are manifest in two environments, including home and school. We decided to combine ADHD combined type and ADHD predominantly hyperactive-impulsive type to one single diagnosis of ADHD predominantly hyperactive-impulsive type. In addition, we used the number of positively scored ADHD criteria, as yielded by the DISC-P as a measure of ADHD severity, irrespective of whether or not subjects fulfilled criteria for one of the ADHD subtypes. Of the 58 patients with a tic disorder 25 (or, 43%) received a diagnosis of no ADHD (mean ADHD severity=4.4; SD=3.3; range=0-11), 18 (or, 31%) of ADHD predominantly hyperactive-impulsive type (5 true ADHD predominantly hyperactive-impulsive type, and 13 ADHD combined type), with a mean ADHD severity of 14.7 (SD=3.4; range=7-18), and 15 (or, 26%) a diagnosis of ADHD predominantly inattentive type (mean ADHD severity=10.1; SD=2.0; range=7-13). Table 1 summarizes data regarding gender, age, medication status, tic severity, type of tic disorder, and severity of ADHD for these categories.

Instruments

Prior to the clinical interview, parents were asked to fill out two parent questionnaires, the CSBQ and the CBCL. All parents of participating patients did complete both questionnaires.

The CSBQ contains 96 items to each of which the parent is asked to respond by indicating whether it does not describe the child (score 0), infrequently describes the child (score 1), or clearly applies to the child (score 2). Parents were asked to base their answers solely on the child's behavior during the last 2 months. On the basis of factor analysis of previously obtained data on a large number of children, five scales have been constructed, together using 66 of the 96 items. These scales have been designated acting-out (scale 1, containing 14 items), social contact problems (scale 2, 13 items), social insight problems (scale 3, 16 items), anxious/rigid (scale 4, 16 items), and stereotypical scale (scale 5, seven items). The CSBQ differentiates between clinical groups and has a good inter-rater and test-retest reliability.[18] The CBCL contains 122 items to which parents have to respond in a way similar to the CSBQ; the CBCL can be divided into eight different scales.

Table 1. *Data regarding age, tic severity, severity of ADHD, type of tic disorder, gender, and medication status for tic disorder patients distinguished by ADHD-status.*

	Tics+ADHD hyperactive-impulsive type		*Tics+ADHD inattentive type*		*Tics-ADHD*	
		N=18		*N*=15		*N*=25
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
Age	10.78	2.56	11.40	2.33	11.20	3.76
Total YGTSS	42.89	20.44	30.73	22.95	33.52	18.62
Motor YGTSS	13.00	3.68	10.80	4.54	11.20	3.76
Vocal YGTSS	8.78	5.14	7.27	6.01	6.32	5.89
Impairment YGTSS	21.11	16.41	12.67	15.80	16.00	13.54
Objective tic severity	21.78	8.02	18.07	9.81	17.52	8.56
ADHD severity	14.7	3.4	10.1	2.0	4.4	3.3
Type of tic disorder	***N***	**%**	***N***	**%**	***N***	**%**
TD	16/18	89	11/15	73	16/25	64
CMT	2/18	11	4/15	27	9/25	36
Gender						
male	13/18	72	12/15	80	18/25	72
female	5/18	28	3/15	20	7/25	28
Medication Status						
medication	13/18	72	13/15	87	12/25	48
no medication	5/18	28	2/15	13	13/25	52

ADHD=attention deficit/hyperactivity disorder; YGTSS=Yale Global Tic Severity Scale; TD=Tourette's disorder; CMT=chronic motor tic disorder

Data analysis

We used two separate multiple analyses of covariance (MANCOVA) to test for differences on the CSBQ and CBCL subscales, respectively, between patients with a tic disorder stratified by ADHD-status and between male and female patients, while using age as a covariate. This was followed by univariate analyses of covariance (ANCOVA), controlling for age, and by pairwise comparisons between the tic disorder groups by means of a Mann-Whitney U Wilcoxon Rank Sum W Test in case of a significant ANCOVA.

Within the patients with a tic disorder as a whole, scores on the subscales of the CSBQ and the CBCL were correlated with measures of ADHD, total CYBOCS scores, and objective tic severity, using Pearson's correlation test. Also, part correlations of the subscales of the questionnaires with ADHD severity, objective tic severity, and severity of obsessions and compulsions separately, each time controlling for the predictive value of the other two measures on the outcome of the questionnaires, were executed. Finally, Pearson's correlation test was used to assess the possible correlation between ADHD severity and objective tic severity, as well as between ADHD severity and CYBOCS scores. All tests of significance used the 0.05 level of significance and were two-tailed.

Results

We compared scores on subscales of the two parent questionnaires between tic disorder patients stratified by ADHD-status and sex, while controlling for the possible effect of age. Regarding CSBQ subscales, we found a significant effect of ADHD-status (Pillai's Trace, $F=3.00$, $p=0.003$), but failed to find an effect of age (Pillai's Trace, $F=1.45$, $p=0.225$) and sex (Pillai's Trace, $F=1.57$, $p=0.189$). With regard to the CBCL subscales, we also encountered a significant effect of ADHD-status (Pillai's Trace, $F=2.19$, $p=0.010$), which was absent for the effect of age (Pillai's Trace, $F=1.58$, $p=0.157$) and sex (Pillai's Trace, $F=0.91$, $p=0.530$). Subsequent ANCOVAS with regard to ADHD-status, while controlling for age, were significant for the CSBQ subscales acting out, social insight problems, and the stereotypical subscale, but failed to reach significance for the social contact problems and anxious/rigid subscales. Also, significant subgroup differences were found with regard to the CBCL subscales attention problems, social problems, as well as the aggressive and delinquent subscales. Of note, patients with a tic disorder with comorbid ADHD hyperactive-impulsive type showed the highest scores on the parent questionnaires, with tic disorder patients with ADHD inattentive type yielding medium scores and tic disorder patients without ADHD consistently having the lowest scores (table 2).

Significance levels of post-hoc pairwise comparisons between the different tic disorder groups are given in table 3. These comparisons demonstrate significant differences between tic disorder patients without ADHD and tic disorder patients with ADHD hyperactive-impulsive type on a considerable number of CSBQ and CBCL subscales, including the CSBQ social insight subscale. Furthermore, table 4 gives an overview of correlation coefficients of ADHD severity, severity of obsessions and compulsions, and tic severity with the different subscales of the parent questionnaires. Also, part correlations of the subscales of the questionnaires with ADHD severity, severity of obsessions and compulsions, and tic severity measures, controlling for the predictive value of the other two measures on the outcome of the questionnaires are represented. ADHD and OCD severity, in contrast to tic severity, correlate with most of the subscales. No correlation, however, was detected between ADHD severity and the CSBQ subscale social contact problems. Finally, no significant correlation was found between ADHD severity and objective tic severity ($r=0.22$, $p=0.103$). In contrast, severity of obsessions and compulsions significantly correlated with both tic severity ($r=0.39$, $p=0.004$) and ADHD severity ($r=0.57$, $p<0.001$).

Table 2. *Scores on (sub)scales of the CSBQ and CBCL across patient groups. F-values and significance levels of the ANCOVA's (controlling for age) are presented as well.*

	Tics+ADHD hyperactive-impulsive type		*Tics+ADHD inattentive type*		*Tics–ADHD*			
	N=18		*N*=15		*N*=25		*Statistics*	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**F**	**P**
CSBQ subscales								
acting out	17.11	5.53	11.13	5.21	7.60	4.75	15.3	<.001
social contact problems	6.17	4.55	5.00	4.42	4.84	4.61	1.39	n.s.
social insight problems	13.89	7.10	11.67	6.52	7.48	5.39	4.04	.024
anxious/rigid scale	11.06	8.00	6.40	4.26	6.04	6.12	3.16	n.s.
stereotypical scale	4.44	3.36	2.60	2.23	1.58	1.69	4.31	.019
CBCL subscales								
attention problems	11.11	4.19	10.67	4.34	6.08	3.93	7.51	.002
social problems	6.00	3.27	5.20	2.91	2.56	2.33	5.03	.011
thought problems	3.78	3.80	3.14	2.93	1.54	2.92	2.80	n.s.
sexual problems	1.06	1.83	0.13	0.35	0.36	0.70	2.91	n.s.
aggressive	17.78	8.21	13.93	6.50	8.54	6.43	9.07	<.001
delinquent	3.72	3.54	4.20	4.48	1.08	1.41	5.17	.009
anxious/depressive	9.17	6.95	7.53	4.36	5.56	5.78	2.99	n.s.
somatic	3.83	3.49	3.62	3.45	3.92	3.61	0.51	n.s.
withdrawn	4.22	3.81	4.80	2.57	3.52	2.76	1.21	n.s.

ADHD=attention deficit/hyperactivity disorder; CSBQ=children's social behavior questionnaire; CBCL=childhood behavior checklist; n.s.=not significant

Table 3. *Significance levels of post-hoc pairwise comparisons (Mann-Whitney U Wilcoxon Rank Sum W Test) between tic disorder patients with or without ADHD subtypes.*

	Tics+ADHD hyperactive-impulsive type versus *Tics+ADHD inattentive type*	*Tics+ADHD hyperactive-impulsive type* versus *Tics–ADHD*	*Tics+ADHD inattentive type* versus *Tics–ADHD*
CSBQ subscales			
acting out	n.s.	<.001	n.s.
social insight	n.s.	.003	n.s.
stereotypical scale	n.s.	.003	n.s.
CBCL subscales			
attention problems	n.s.	.001	.002
social problems	n.s.	.001	.009
aggressive	n.s.	.003	n.s.
delinquent	n.s.	.005	.008

ADHD=attention deficit/hyperactivity disorder; CSBQ=children's social behavior questionnaire; CBCL=childhood behavior checklist; n.s.=not significant

Table 4. *Part correlations of the scales of the questionnaires with either ADHD severity, severity of obsessions and compulsions (OCD severity), or tic severity, controlling for the predictive value of OCD, tic, and ADHD severity, respectively, on the outcome of the questionnaires. Also, uncontrolled correlation coefficients are given (first columns). Correlations are only represented if they are greater than .30 and if p<0.05.*

	ADHD severity	*ADHD severity (part)*	*Tic severity*	*Tic severity (part)*	*OCD severity*	*OCD severity (part)*
CSBQ subscales						
acting out	.71	.61			.40	
social contact problems						
social insight problems	.58	.50			.33	
anxious/rigid scale	.43		.40		.57	.31
stereotypical scale	.39		.42		.48	
CBCL subscales						
attention problems	.62	.52			.38	
social problems	.59	.45			.44	
thought problems	.39		.46		.72	.49
sexual problems					.30	
aggressive	.61	.54			.32	
delinquent	.44	.38				
anxious/depressive	.38				.47	
somatic					.31	
withdrawn						

ADHD=attention deficit/hyperactivity disorder; OCD=obsessive-compulsive disorder; CSBQ=children's social behavior questionnaire; CBCL=childhood behavior checklist;

Discussion

The overall high scores on the CSBQ and CBCL subscales of the tic disorder patients, when compared to what was previously found in healthy control subjects who had never been in contact with mental health services,[18] demonstrates the significance and magnitude of behavior and social problems of clinically referred children and adolescents with a tic disorder and is an indication of the broad range of possibly associated problems in referred tic disorders. In accordance with previous studies,[2] more than half of our patients with a tic disorder fulfilled criteria for ADHD. A first indicator of the importance of ADHD for behavioral and social problems in children with a tic disorder yields a comparison of parent rating scores between tic disorder patients with ADHD primarily hyperactive-impulsive type, tic disorder patients with ADHD primarily inattentive type, and tic disorder patients without ADHD. In addition, the results of the correlations between tic and ADHD severity, respectively, with the parent questionnaires, clearly demonstrate that it is the severity of ADHD, and not the severity of the tics per se, which determines the magnitude of many associated features. This study specifically examined the contribution of tic symptom severity versus ADHD severity and severity of obsessions and compulsions, to the outcomes of the parent rating scales. Future studies should address the contribution of other comorbid disorders such as mood and anxiety disorders to social-behavioral functioning in children and adolescents with a tic disorder, which may play a role alongside ADHD and OCD. However,

irrespective of the role of these other comorbidities, the present data unequivocally indicate an absent association of indices of social functioning and behavior problems with tic severity.

The existing association between severity of tics and the stereotypical and anxious/rigid subscale of the CSBQ seems to reflect the incorporation of items that measure repeated motor movements and obsessive thoughts, respectively, in these CSBQ subscales. The positive correlation between tic severity and the CBCL subscale thought problems is largely due to obsessive-compulsive symptoms, as evidenced by the clear correlation of this subscale with severity of obsessions and compulsions, and the absent correlation with tic severity when controlling for ADHD and CYBOCS scores. Thus, association with obsessive-compulsive symptoms seems to be tic-specific. In contrast, independently from the severity of the tic disorder, the degree of comorbid ADHD predicts the presence of associated features that are well beyond the defining symptoms of ADHD. A striking example is the association with the CSBQ subscale social insight problems. Parents respond to items like 'does not fully understand what is being said to him/her, i.e. tends to miss the point', 'is exceptionally naive; believes anything you say', 'does not understand jokes', or 'frequently says things which are not relevant to the conversation'. These social insight problems, albeit no part of formal DSM-IV symptomatology, nonetheless constitute an important problem area in clinical practice, given the substantial impact for both patients and their parents of social dysfunctioning on such diverse areas like self-esteem, school functioning, and managing the children at home. Previous experience with the CSBQ indicates similar ADHD-associated social problems in ADHD cases without tics,[19] which makes it unlikely that these are specifically linked to ADHD in tic disorder patients. However, future studies should compare the relative contribution of ADHD symptoms to social functioning in patients with ADHD with and without tics.

The exact relationship between tic disorders and associated mental disorders is an issue of considerable controversy. There has been much debate about the 'broadness' of the phenotypic expression of the gene(s) responsible for tic disorders. Comings and Comings[21] concluded on the basis of their extensive family studies a wide range of behavioral disorders, including ADHD, to be an integral part of tic spectrum disorders, whereas the researchers of the Yale Child Study Center presented evidence against a simple genetic relationship between tic disorders and ADHD.[22] Of particular note is the absent correlation between ADHD and tic severity in our data. This finding speaks against the view of TD as a broad disorder in which behavioral problems form an intrinsic part of the disorder. Even though some of the reported attentional symptoms might represent executive dysfunction,[23] they still do not appear to correlate with tic severity.

Other authors pointed to the possibility of an association of tic disorders with pervasive developmental disorders (PDD). Social contact problems, as evidenced by little or no need for contact with others, form the clinical hallmark of PDD. In the literature, several coincident cases of TD and PDD have been described.[20,24,25] In addition, Comings and Comings[26] presented a number of cases of TD probands with autistic relatives and expressed the opinion that some cases of PDD are due to

the TD gene(s). Indeed, in the present study, compared to healthy controls, the tic disorder patients obtained higher scores on both scale 2 of the CSBQ, designated social contact problems, as well as on the CBCL withdrawn subscale. However, these subscales did not correlate with tic severity. This speaks against a direct relationship between tic disorders and PDD. Stern et al.[24] state that in their TD clinic, no striking impression of even an anecdotal association of TD with PDD exists and suggest an alternative explanation, namely a TD/tic phenocopy in those PDD who present with tics. Our correlation results seem to support the view of the latter authors.

In conclusion, the results of the present study confirm that clinically referred patients with tic disorders show a wide range of behavioral and social problems. No support, however, was found for a direct link of these associated features with tic disorders. Our finding that, apart from obsessions and compulsions, these associated features seem secondary to comorbid ADHD, is remarkably consistent with a previous report that assessed neuropsychiatric correlates in children with TD with and without ADHD[27] as well as with a recent study reporting that it is the comorbid ADHD, in contrast with tic severity, that is highly associated with disruptive behavior and functional impairment in children with TD.[28]

In the present study, we relied on patients with a tic disorder that had been referred to an outpatient clinic for child and adolescent psychiatry. Ascertainment bias may thus have influenced our study results. The results should be viewed with caution, since it may well be that our patients had been referred to mental health services due to behavioral problems, rather than because of their tics. However, even against this background, we found evidence against an intrinsic relationship between tic disorders and associated phenomena, including ADHD.

References

1. King, R. A., & Scahill L., (2001). Emotional and behavioral difficulties associated with Tourette syndrome. *Advances in Neurology.* 85, 79-88.
2. Kadesjo, B., & Gillberg, C. (2000). Tourette's disorder: epidemiology and comorbidity in primary school children. *Journal of the American Academy of Child and Adolescent Psychiatry.* 39, 548-555.
3. Sheppard, D. M., Bradshaw, J. L., Purcell, R., & Pantelis, C. (1999). Tourette's and comorbid syndromes: obsessive compulsive and attention deficit hyperactivity disorder. A common etiology? *Clinical Psychology review.* 19, 531-552.
4. Leckman, J. F., Grice, D. E., Barr, L. C., de Vries, A. L., Martin, C., Cohen, D. J., McDougle, C. J., Goodman, W. K., & Rasmussen, S.A. (1994). Tic-related versus non-tic-related obsessive compulsive disorder. *Anxiety.* 1. 208-215.
5. Comings, D. E., & Comings, B.G., (1987). A controlled study of Tourette syndrome. IV. Obsessions, compulsions, and schizoid behaviors. *American Journal of Human Genetics.* 41, 782-803.
6. Coffey, B. J., Biederman, J., Smoller, J. W., Geller, D. A., Sarin, P. Schwartz, S., & Kim, G. S. (2000a). Anxiety disorders and tic severity in juveniles with Tourette's disorder. *Journal of the American Academy of Child and Adolescent Psychiatry.* 39, 562-568.
7. Coffey, B. J., Biederman, J., Geller, D. A., Spencer, T. J., Kim, G. S., Bellordre, C. S., Frazier, J. A., Cradock, K., & Magovcevic, M. (2000b). Distinguishing illness severity from tic severity in children and adolescents with Tourette's disorder. *Journal of the American Academy of Child and Adolescent.* 39, 556-561.
8. Champion, L. M., Fulton, W. A., & Shady, G. A. (1988). Tourette syndrome and social functioning in a Canadian population. *Neuroscience and biobehavioral reviews.* 12, 255-257.
9. Stokes, A., Bawden, H. N., Camfield, P. R., Backman, J. E., & Dooley, J. M. (1991). Peer problems in Tourette's disorder. *Pediatrics.* 87, 936-942.
10. Kurlan, R., Daragjati, C., Como, P. G., McDermott, M. P., Trinidad, K. S., Roddy, S., Brower, C. A., & Robertson, M. M. (1996). Non-obscene complex socially inappropriate behavior in Tourette's syndrome. *Journal of Neuropsychiatry and Clinical Neuroscience.* 8, 311-317.
11. Singer, H. S., & Rosenberg, L. A. (1989). Development of behavioral and emotional problems in Tourette syndrome. *Pediatric Neurology.* 5, 41-44.
12. Achenbach, T. M., & Edelbrock, C. S. (1981). Behavioral problems and competencies reported by parents of normal and disturbed children aged four through 16. *Monographs of the Society for Research in Child Development.* 46, 1-82.
13. Rosenberg, L. A., Brown, J., & Singer, H. S. (1995). Behavioral problems and severity of tics. *Journal of Clinical Psychology.* 51, 760-767.
14. Barkley, R. A. (1997). Behavioral inhibition, sustained attention, and executive functions: constructing a unifying theory of ADHD. *Psychological Bulletin.* 121, 65-94.
15. Bawden, H. N., Stokes, A., Camfield, C. S., Camfield, P. R., & Salisbury, S. (1998). Peer relationship problems in children with Tourette's disorder or diabetes mellitus. *Journal of Child Psychology and Psychiatry.* 39, 663-668.
16. Dykens, E., Leckman, J., Riddle, M., Hardin, M., Schwartz, S., & Cohen, D. (1990). Intellectual, academic, and adaptive functioning of Tourette syndrome children with and without attention deficit disorder. *Journal of Abnormal Child Psychology.* 18, 607-615.
17. Luteijn, E., Jackson, S., Volkmar, F. R., & Minderaa, R. B. (1998). Brief report: the development of the Children's Social Behavior Questionnaire: preliminary data. *Journal of Autism and Developmental Disorders.* 28. 559-565.
18. Luteijn, E., Luteijn, F., Jackson, S., Volkmar, F., & Minderaa, R. (2000a). The children's Social Behavior Questionnaire for milder variants of PDD problems: evaluation of the

psychometric characteristics. *Journal of Autism and Developmental Disorders.* 30, 317-330.

19. Luteijn, E. F., Serra, M., Jackson, S., Steenhuis, M. P., Althaus, M., Volkmar, F., & Minderaa, R. (2000b). How unspecified are disorders of children with a pervasive developmental disorder not otherwise specified? A study of social problems in children with PDD-NOS and ADHD. *European Child and Adolescent Psychiatry.* 9, 168-179.

20. Scahill, L., Riddle, M. A., McSwiggin-Hardin, M., Ort, S. I., King, R. A., Goodman, W. K. Cicchetti D., & Leckman, J. F. Children's Yale-Brown Obsessive Compulsive Scale: reliability and validity. (1997). *Journal of the American Academy of Child and Adolescent Psychiatry*. 36, 844-852.

21. Comings, D. E., & Comings, B.G., (1990). A controlled family history study of Tourette's syndrome, I: Attention-deficit hyperactivity disorder and learning disorders. *Journal of Clinical Psychiatry.* 51, 275-280.

22. Pauls, D. L., Hurst, C. R., Kruger, S. D., Leckman, J. F., Kidd, K. K., & Cohen, D. J. (1986). Gilles de la Tourette's syndrome and attention deficit disorder with hyperactivity. Evidence against a genetic relationship. *Archives of General Psychiatry.* 43, 1177-1179.

23. Muller, S. V., Johannes, S., Wieringa, B., Weber, A., Muller-Vahl, K., monitoring and response inhibition in patients with Gilles de la Tourette Syndrome and co-morbid obsessive compulsive disorder. *Behavioral Neurology.* 14, 29-37.

24. Stern, J. S., & Robertson, M. M. (1997). Tics associated with autistic and pervasive developmental disorders *Neurologic Clinics.* 15, 345-355.

25. Baron-Cohen, S., Scahill, V. L., Izaguirre, J., Hornsey, H., & Robertson, M. M., (1999). The prevalence of Gilles de la Tourette syndrome in children and adolescents with autism: a large scale study. *Psychological Medicine.* 29, 1151-1159.16.

26. Comings, D. E., & Comings, B.G., (1991). Clinical and genetic relationships between autism-pervasive developmental disorder and Tourette syndrome: a study of 19 cases. *American Journal of Medical Genetics.* 39, 180-191.

27. Spencer, T., Biederman, J., Harding, M., O'Donnell, D. Wilens, T., Faraone, S., Coffey, B., & Geller, D. (1998). Disentangling the overlap between Tourette's disorder and ADHD. *Journal of Child Psychology and Psychiatry.* 39, 1037-1044.

28. Sukhodolsky, D. G., Scahill, L., Zhang, H., Peterson, B. S., King, R. A., Lombroso, P. J., Katsovich, L., Findley, D., & Leckman, J. F. (2003). Disruptive behavior in children with Tourette's syndrome: association with ADHD comorbidity, tic severity, and functional impairment. *Journal of the American Academy of Child and Adolescent Psychiatry.* 42, 98-105.

Chapter 3

Elevated D8/17 expression on B lymphocytes, a marker of rheumatic fever, measured with flow cytometry in tic disorder patients

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Abstract

Objective: *Elevated D8/17 expression on B lymphocytes is a known susceptibility marker of rheumatic fever. Previous studies have reported higher than usual D8/17 expression on B lymphocytes of patients with tic disorders. The purpose of this study was to assess D8/17 expression on B lymphocytes of tic disorder patients by using an objective method in which no operator variability was involved.*

Methods: *D8/17 expression on B lymphocytes was assessed with flow cytometry by using an immunoglobulin M (IgM) monoclonal D8/17-specific antibody in an unselected group of Dutch patients with tic disorders (N=33) and healthy volunteers (N=20). Binding of this monoclonal antibody was compared with binding of an irrelevant IgM monoclonal antibody, and the shift in mean fluorescence intensity of the D8/17-specific antibody compared to that of the irrelevant IgM monoclonal antibody was used as a measure of D8/17 overexpression. For the patients, Yale Global Tic Severity Scale scores were used to assess disease severity.*

Results: *D8/17 overexpression in the patient group (mean=16.8 arbitrary units, SD=30.5) was significantly higher than in the comparison group (mean=3.2, SD=3.0). A significant minority of the patients (N=13, 39.4%), however, had their levels of D8/17 overexpression within the range of that of the healthy comparison subjects. Flow cytometric analysis did not indicate a separate subpopulation of D8/17-positive B cells.*

Conclusion: *These data confirm the utility of D8/17 B cell overexpression as a peripheral blood marker in patients with tic disorders and are compatible with a streptococcus-related pathogenesis for at least a subgroup of patients with tic disorders.*

Introduction

The pathogenesis of childhood-onset tic disorders is not well understood. In addition to genetic factors, autoimmunity may be involved in these disorders.[1] Sydenham's chorea has been postulated as a medical model for tic disorders.[2] A possible indicator of autoimmunity is the greater than usual binding of a D8/17-specific monoclonal antibody to B lymphocytes.[3-4] D8/17-specific monoclonal antibody is a mouse immunoglobulin M (IgM) monoclonal antibody originally prepared from fusions of spleen cells from mice that had been repeatedly immunized with isolated human B cells obtained from patients with rheumatic fever or rheumatic heart disease.[5] D8/17-specific monoclonal antibody has been reported to bind to a small percentage of B lymphocytes in normal comparison subjects (averaging 5%-7%), but in patients with rheumatic fever, the percentage of D8/17-positive B lymphocytes was found to be much higher (mean=33.5%).[5] It has been proposed that an individual could be classified as "D8/17-positive" when his D8/17 B cell expression exceeded the mean plus one standard deviation of that of the healthy comparison subjects (that is, 12%). The D8/17-positive status has been suggested as a susceptibility marker for rheumatic fever, a well-known complication of infections with group A beta-hemolytic streptococci; 60%-100% of the subjects with rheumatic fever studied so far have been reported to be D8/17-positive.[6-11]

Two research centers reported elevated D8/17 expression on B cells in patients with tic disorders. A National Institute of Mental Health group[4] investigated 27 children who fulfilled the criteria for pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections (PANDAS), nine children with Sydenham's chorea, and 24 healthy comparison subjects. The children with PANDAS represent a separate subgroup of patients from the spectrum of illness encompassing Tourette's syndrome and obsessive-compulsive disorder (OCD), whose tics and obsessive-compulsive symptoms are shown to arise in response to beta-hemolytic streptococcal infections. They found that 85% of the children with PANDAS, 89% of the children with Sydenham's chorea, and only 17% of the comparison subjects were D8/17-positive.[4] Murphy et al.[3] compared 31 patients with childhood-onset OCD, Tourette's syndrome, or chronic tic disorders with 21 healthy comparison subjects and found that all patients and only one comparison subject were D8/17-positive. They suggested that D8/17-positivity may serve as a susceptibility marker for the development of tics or obsessive-compulsive symptoms.

In both studies, D8/17 expression on B cells was assessed by indirect immunofluorescence, after which D8/17-positive cells were scored by means of fluorescence microscopy. In the study of Murphy et al.,[3] the investigators were not always completely blind to the subject's disease status. Given the subjective nature of the use of indirect immunofluorescence, we intended to improve the methodology of assessing D8/17 B cell expression by applying a method in which no operator variability was involved, and controlling for nonspecific binding.

We report here on D8/17 expression on B lymphocytes in an unselected group of Dutch patients with tic disorders and in a group of healthy volunteers by means of flow cytometry. In addition, we correlated D8/17 expression with disease severity as measured by the Yale Global Tic Severity Scale.[12]

Methods

Subjects

Two groups of subjects were chosen for the study: healthy volunteers and patients with a definite tic disorder per the Tourette's Syndrome Research Criteria.[13] According to these criteria, observable tics must be present during the clinical interview to allow for study entry. The aim and procedure of the study were fully explained to the subjects before written consent was requested. If the subjects were under 18, the parents were informed as well, and the written informed consent of the parents and the subject's assent were obtained.

None of the comparison subjects or their families was reported to show symptoms of a tic disorder or any other neuropsychiatric disorder. Another exclusion criterion for the comparison group was any known history or family history of an autoimmune disorder, including a history of rheumatic fever. Twenty healthy volunteers entered the study (13 male, seven female), ranging in age from 8 to 32 years (mean=13.9, SD=5.0).

The patients were recruited from the outpatient clinic of the Child and Adolescent Psychiatry Center or from members of the Tourette's syndrome patients' association in the Netherlands. None of the patients reported a history of autoimmune disorder, including a history of rheumatic fever. Thirty-three patients entered the study (19 male, 14 female), ranging in age from 6 to 59 years (mean=20.6, SD=14.5). The presence of psychiatric disorders other than autism was not an exclusion criterion. Psychiatric screening was performed by two of the authors, an experienced physician in the field of tic disorders (P.J.H.) and a board-certified child psychiatrist (P.W.T.). Blood was sampled from the subjects within a week of the psychiatric screening.

To assess tic severity, we used the Yale Global Tic Severity Scale,[12] which consists of separate scales for motor and vocal tics complemented with a separate rating of impairment. The mean total Yale Global Tic Severity Scale score for the patients was 37.81 (SD=24.33, range=5-93), with a mean motor score of 13.44 (SD=5.09, range=5-24), a mean vocal score of 6.88 (SD=6.41, range=0-22), and a mean impairment score of 17.5 (SD=16.1, range=0-50). Ten of the patients were taking psychotropic medication, either clonidine (N=3) or various antipsychotic agents (N=7); 23 were free of medication.

Immunology

Both groups were assessed for D8/17 B cell expression by an examiner (J.B.) who was blind to subject status. Blood was collected in acid citrate dextran tubes (acid citrate dextran solution B tubes, Terumo Europe, Leuven, Belgium), and the blood-

staining procedure was carried out on fresh cells. Flow cytometric analysis was performed within 24 hours.

Staining was performed by adding 30 µl of an irrelevant IgM monoclonal antibody, MOC32, directed against neuroendocrine antigens of epithelial origin of small cell lung cancer cells (tube A) or 30 µl of the D8/17-specific monoclonal antibody (tube B), to 100 µl of whole blood. Both the D8/17-specific monoclonal antibody and MOC32 were available in a concentration of 150 µg/ml and were used in an undiluted form. After incubation for 1 hour at 4° C, the suspension was washed with 2 ml of phosphate-buffered saline with 0.5% bovine serum albumin (Sigma Aldrich, Zwijndrecht, the Netherlands) and centrifuged at 2 500 rpm for 2 minutes. To both pellets, 5 µl of phycoerythrin-conjugated CD19 (IQP, Groningen, the Netherlands) as well as 5 µl of fluorescein isothiocyanate-conjugated goat anti-mouse IgM (SBA, Birmingham, Ala.) was added for half an hour at room temperature. Phycoerythrin-conjugated CD19 was used as a marker of the total B cell subpopulation, whereas the fluorescein isothiocyanate-conjugated goat anti-mouse IgM was able to detect binding of the D8/17-specific monoclonal antibody and of the irrelevant monoclonal antibody, respectively. After incubation, the cells were lysed with 3 ml of fluorescence-activated cell sorter lysing solution (BD, Leiden, the Netherlands) for 10 minutes, centrifuged, and washed. The pellets were resuspended in 100 µl of phosphate-buffered saline with 0.5% bovine serum albumin and stored at 4° C until measured on a FACSstar (Becton Dickinson, Woerden, the Netherlands).

Measuring was performed by placing a gate around the CD19-positive B cells and counting 2 000 cells. As shown in figure 1, intersecting perpendicular lines were placed to define the R2 quadrant with the comparison IgM monoclonal antibody at 1% (top row) in order to examine D8/17-positive B cells (bottom row). Overexpression of D8/17 B cells was calculated by subtracting the mean fluorescence intensity of the comparison monoclonal antibody from the mean fluorescence intensity of the D8/17-specific monoclonal antibody. Negative values of this shift in mean fluorescence intensity were set at 0. Individuals were classified as D8/17-negative (<95th percentile of the D8/17 B cell overexpression of the comparison subjects) or D8/17-positive (≥95th percentile of the D8/17 B cell overexpression of the comparison subjects).

Data analysis

We used the Mann-Whitney U test to test differences between the D8/17 B cell overexpression of patients and of comparison subjects. To rule out medication effects, we also tested differences between D8/17 B cell overexpression of patients who were not taking medication and of healthy comparison subjects, by means of the Mann-Whitney U test. Spearman's rank correlation test was used to assess the relationship between D8/17 overexpression and the total as well as motor and phonic scores of the Yale Global Tic Severity Scale.[12]

To assess the relationship between D8/17 B cell overexpression and patient age, we used Spearman's rank correlation test. To investigate possible differences

between D8/17 B cell overexpression and sex in patients, we used the Mann-Whitney U test. All tests of significance used the 0.05 level of significance and were two-tailed.

Results

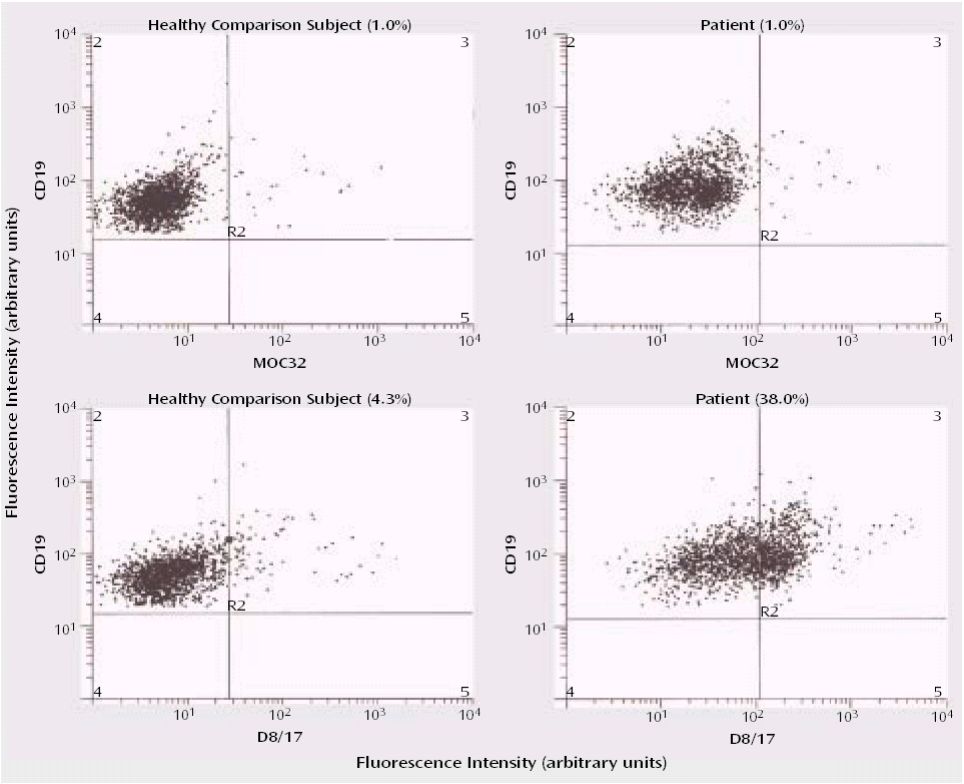
We assessed D8/17 overexpression on B cells by means of flow cytometry in subjects with tic disorders and a healthy comparison group. Figure 1 (lower right corner) shows a typical flow cytometric picture of a D8/17-positive patient with a relatively large overexpression of D8/17 compared to the binding of the irrelevant monoclonal antibody. Also, the results obtained with a comparison subject are shown, demonstrating a slight overexpression of D8/17. Figure 1 shows that the population of B cells stained with D8/17-specific monoclonal antibody is fairly homogeneous: no separate subpopulation of D8/17-positive cells can be distinguished, despite the marked shift in mean fluorescence intensity of the D8/17-specific antibody compared to the expression of the comparison monoclonal antibody.

Median D8/17 B cell overexpression was significantly (Mann-Whitney U=121.0, df=51, $p<0.001$) higher in the patient group (mean=16.8 arbitrary units, SD=30.5, median=11.6, range=0-179.6) than in the comparison group (mean=3.2, SD=3.0, median=2.8, range=0-10.1). For the total group, the median shift in mean fluorescence intensity was 6.3 (arbitrary units) (mean=11.8, SD=24.9, range=0-179.6). Figure 2 shows D8/17 overexpression on B cells in the total group, in tic disorder patients, and in comparison subjects. A comparison of the patient group that was not taking medication (mean=10.6, SD=7.0, median=10.6) with the healthy comparison subjects also revealed significant differences in D8/17 B cell overexpression (Mann-Whitney U=97.5, df=41, p=0.001).

When the cutoff point for an individual's D8/17-positivity was set at 10.0 (at the 95th percentile of the D8/17 B cell overexpression of the comparison subjects), one (5%) of the comparison subjects was D8/17-positive, whereas 20 (60.6%) of the patients were D8/17-positive.

Furthermore, we tested whether the level of D8/17 overexpression correlated with the severity of the tic disorder. There was no statistically significant positive correlation between the level of D8/17 expression and the severity of tics as measured by the motor (r=0.24, df=31, p=0.18), phonic (r=-0.20, df=31, p=0.25), or total (r=0.16, df=31, p=0.37) score on the Yale Global Tic Severity Scale. There was no correlation between D8/17 expression and age. The mean D8/17 expression of the male patients did not differ from that of the female patients (Mann-Whitney U=117.0, df=31, p=0.58).

Figure 1. *Flow cytometric analysis of D8/17 expression on B lymphocytes in a healthy comparison subject and in a tic disorder patient with higher than normal D8/17 expression.**



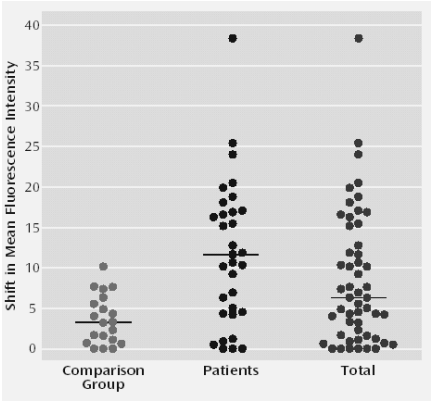
*Each dot represents one cell. Both axes refer to fluorescence intensity reflecting the magnitude of the respective antibody binding (D8/17-specific monoclonal antibody, MOC32, or CD19) of each cell. The top two panels show expression on the B cells of an irrelevant monoclonal antibody (MOC32). The two bottom panels show expression of D8/17-specific monoclonal antibody, showing that the cell population is fairly homogeneous. Therefore, measurement of cells gated to the R2 quadrant is not entirely precise, indicating measurement of shift in mean fluorescence intensity is a more accurate measure of D8/17 B cell overexpression. The area of specific binding is defined as the area in which less than 1% of the B cells bind to the irrelevant monoclonal antibody MOC32.

Discussion

As a group, the patients with a tic disorder showed significantly higher D8/17 overexpression on B lymphocytes than did the healthy volunteers, thus confirming the findings of earlier reports. However, no support could be found for the distinction of a separate subpopulation of D8/17-positive B cells. Given this finding, it is unclear how D8/17-positive B cells could be distinguished and counted manually by means of indirect immunofluorescence, as described in the reports of Murphy et al.[3] and Swedo et al.[4]

Apparently, contrary to earlier concepts (e.g., reference 5), D8/17-positive patients do not show an elevated number of D8/17-positive B cells but, rather, show elevated D8/17 expression on the B cell population as a whole, which can accurately be measured by calculation of the shift in mean fluorescence intensity of the D8/17-specific antibody compared to that of the irrelevant IgM monoclonal antibody.

Figure 2. *Levels of overexpression of D8/17 antigen on B lymphocytes in 20 healthy comparison subjects, 33 tic disorder patients, and the total group.**



*Shift in mean fluorescence intensity of the D8/17-specific antibody compared to that of the irrelevant immunoglobulin M (IgM) monoclonal antibody. Horizontal lines represent median values.

A significant minority of patients (N=13, 39.4%), however, had levels of D8/17 overexpression that fell within the range of that of the healthy comparison subjects. In this respect, our results differ from those of Murphy et al.[3] who reported, using a different method, that all of their 31 patients had elevated levels of D8/17 expression. However, a recent study in an Afrikaner population in South Africa[14] also reported an overlap in D8/17 expression between healthy comparison subjects and OCD patients. Ten out of 11 patients with OCD and 14 out of 22 comparison subjects were reported to be D8/17-positive (defined as having 12% or more D8/17-positive B lymphocytes) in that study as determined by immunofluorescence microscopy. If the cutoff point for D8/17-positivity in that study had been defined as the 95th percentile for the comparison subjects, the results would have been virtually identical to our data.

The present study is, to our knowledge, the first using flow cytometry with a comparison IgM monoclonal antibody for assessing D8/17 expression on B lymphocytes. Flow cytometry is an objective method in which no operator

variability is involveld that allows for analysis of many more cells than is possible by counting D8/17-positive cells by using a fluorescence microscope. Previously, Chapman and coworkers[15] also described a flow cytometric assay for assessing D8/17 B cell expression in patients with Tourette's syndrome or OCD, but their method clearly differed from ours, since they did not control binding of the D8/17-specific monoclonal antibody for nonspecific binding by using an irrelevant monoclonal antibody. Therefore, they measured a percentage of D8/17-positive B cells instead of using a shift in mean fluorescence intensity of the D8/17-specific antibody compared to that of the irrelevant IgM monoclonal antibody as a measure of D8/17 overexpression. Chapman and coworkers compared the results of their flow cytometric assay with the results of cell counting using immunofluorescence microscopy and found a significant but not perfect correlation (r=0.82). In their study, D8/17 B cell expression of patients and comparison subjects also clearly overlapped, irrespective of the method of assessment. Those data are in accordance with our data, but they clearly differ from the earlier reports of Swedo et al.[4] and Murphy et al.[3]

The characterization of the antigen recognized by the D8/17-specific monoclonal antibody as well as the pathogenetic meaning of D8/17 B lymphocyte overexpression awaits further study. The D8/17-specific monoclonal antibody has not only been found to bind to B cell surface structures, but also to diverse tissue sites in humans such as the myocardium, smooth muscle, skeletal muscle, and epithelium cells. The monoclonal antibody appears to bind to the cytoskeletal helical coil/coiled structures myosin and tropomyosin. It is of interest that the D8/17-specific monoclonal antibody was also found to bind to streptococcal M proteins.[16] Because of such cross-reactivities, structural similarities may exist between the cytoskeletal proteins myosin and tropomyosin, surface antigens present on a subset of B cells, and streptococcal M proteins. It is unclear how these findings relate to the model of molecular mimicry that is thought to lead to the symptom complex of rheumatic fever. However, elevated D8/17 expression may point to an individual's susceptibility to experience autoimmune complications in the aftermath of streptococcal infections. Apparently, some individuals with increased D8/17 expression develop rheumatic fever, whereas others are prone to the emergence of tic disorders. On the other hand, there are considerable numbers of patients with tic disorders who show D8/17 expression in the normal range. It is not known whether D8/17-positive patients with Tourette's syndrome represent a distinct subgroup within the spectrum of tic disorders in which autoimmunity may be involved, or whether alternative disease mechanisms may be involved in D8/17-negative patients with tic disorders. Carefully comparing D8/17-positive with D8/17-negative patients, both clinically and serologically, as well as with regard to treatment response, should identify possible differences between these patients.

The specificity of D8/17-positivity for child neuropsychiatric disorders remains to be elucidated, since elevated D8/17 B cell binding in autism has also been reported.[17] In this group, a positive correlation was found between the percentage of D8/17-positive B cells and repetitive behavior, the hallmark of tic disorders. We

found no correlation between D8/17 B cell expression and tic severity as measured by the Yale Global Tic Severity Scale. In contrast to those of autism, the symptoms of tic disorders are liable to fluctuate, which can possibly obscure this correlation in patients with tic disorders, since it is not known whether percentages of D8/17 expression reflect such fluctuations.

Furthermore, the ethnic background of the patient sample might be a relevant factor. For example, 90%-100% of the rheumatic fever patients in the United States (of unspecified ethnic origin) were found to have elevated D8/17 B cell expression, whereas it was found in only 66% of the rheumatic fever subjects in India.[18] Specific regional susceptibility markers for the latter population have been found.[19] It is therefore of interest to investigate whether different susceptibility markers also exist in tic disorder patients across different regions. To our knowledge, the present study is the first report on D8/17 B cell overexpression in Western Europe. Clearly, more data are needed concerning D8/17 B cell expression in healthy comparison subjects across different population groups with the use of objective measures.

Much work remains to be done in order to elucidate the role of autoimmunity in tic disorders, especially with regard to the role of previously reported auto-antibodies.[20-22] To further investigate the usefulness of the proposed concept of PANDAS, studies comparing Tourette's syndrome subjects who fulfill the criteria for PANDAS with non-PANDAS subjects must be performed.

To conclude, the present finding of elevated D8/17 B cell overexpression by an objective method in a psychiatric population is highly intriguing. Elevated D8/17 B cell expression might not only serve as an objective blood marker for at least a subgroup of the tic disorder spectrum, but moreover, it points towards a streptococcus-related pathogenesis with potentially promising implications for further fruitful research, possibly leading to more effective future interventions. Apart from providing insight in the possible pathophysiology, a blood marker may stimulate research in other fields. For example, if elevated D8/17 B cell expression is a reliable and stable marker in a subgroup of patients, it could facilitate the composition of homogeneous subgroups for genetic studies.

References

1. Hallett JJ, Kiessling LS: Neuroimmunology of tics and other childhood hyperkinesias. *Neurol Clin* 1997; 15:333-344

2. Swedo SE: Sydenham's chorea. A model for childhood autoimmune neuropsychiatric disorders. *JAMA* 1994; 272:1788-1791

3. Murphy TK, Goodman WK, Fudge MW, Williams RC, Jr., Ayoub EM, Dalal M, Lewis MH, Zabriskie JB: B lymphocyte antigen D8/17: a peripheral marker for childhood-onset obsessive-compulsive disorder and Tourette's syndrome? *Am J Psychiatry* 1997; 154:402-407

4. Swedo SE, Leonard HL, Mittleman BB, Allen AJ, Rapoport JL, Dow SP, Kanter ME, Chapman F, Zabriskie J: Identification of children with pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections by a marker associated with rheumatic fever. *Am J Psychiatry* 1997; 154:110-112

5. Khanna AK, Buskirk DR, Williams RC, Jr., Gibofsky A, Crow MK, Menon A, Fotino M, Reid HM, Poon-King T, Rubinstein P: Presence of a non-HLA B cell antigen in rheumatic fever patients and their families as defined by a monoclonal antibody. *J Clin Invest* 1989; 83:1710-1716

6. Kilasoniia LO, Chumburidze VB, Tatishvili NI, Polianskaia IS, Tsitlanadze VG: A new immunogenetic marker of rheumatism. *Klin Med* (Mosk) 1989; 67:64-66

7. Shostak NA, Anokhin VN, Kazakova TV, Morozova EI, Zolkina IV, Voilokova RI: Detection of B-cell marker of rheumatism using monoclonal antibodies D8/17 in families of patients with rheumatism (report 1). *Revmatologiia* (Mosk) 1990; 27-31

8. Taneja V, Mehra NK, Reddy KS, Narula J, Tandon R, Vaidya MC, Bhatia ML: HLA-DR/DQ antigens and reactivity to B cell alloantigen D8/17 in Indian patients with rheumatic heart disease. *Circulation* 1989; 80:335-340

9. Shostak NA, Anokhin VN, Zolkina IV, Morozova EI, Kazakova TV: Detection of B-cell marker of rheumatism using monoclonal antibodies D8/17 in the families of patients with rheumatism. *Revmatologiia* (Mosk) 1991; 24-26

10. Shostak NA: The diagnostic significance of the surface B-cell marker carrier state in arthritis and other manifestations of rheumatic fever. *Ter Arkh* 1991; 63:49-52

11. Rodriguez RS, Ontiveros P, Torres S, Khanna AK, Buskirk DR, Zabriskie JB: Presence of a non-HLA antigen in B-lymphocytes from patients with rheumatic fever and their relatives defined using monoclonal antibodies. *Bol Med Hosp Infant Mex* 1990; 47:313-317

12. Leckman JF, Riddle MA, Hardin MT, Ort SI, Swartz KL, Stevenson J, Cohen DJ: The Yale Global Tic Severity Scale: initial testing of a clinician-rated scale of tic severity. *J Am Acad Child Adolesc Psychiatry* 1989; 28:566-573

13. Kurlan R: Diagnostic criteria for genetic studies of Tourette syndrome. *Arch Neurol* 1997; 54:517-518

14. Niehaus DJ, Knowles JA, van Kradenberg J, du TW, Kaminer D, Seedat S, Daniels W, Cotton M, Brink P, Beyers AD, Bouic P, Chapman F, Zabriskie JB, Stein DJ: D8/17 in obsessive-compulsive disorder and trichotillomania. *S Afr Med J* 1999; 89:755-756

15. Chapman F, Visvanathan K, Carreno-Manjarrez R, Zabriskie JB: A flow cytometric assay for D8/17 B cell marker in patients with Tourette's syndrome and obsessive compulsive disorder. *J Immunol Methods* 1998; 219:181-186

16. Kemeny E, Husby G, Williams RC, Jr., Zabriskie JB: Tissue distribution of antigen(s) defined by monoclonal antibody D8/17 reacting with B lymphocytes of patients with rheumatic heart disease. *Clin Immunol Immunopathol* 1994; 72:35-43

17. Hollander E, Delgiudice-Asch G, Simon L, Schmeidler J, Cartwright C, Decaria CM, Kwon J, Cunningham-Rundles C, Chapman F, Zabriskie JB: B lymphocyte antigen D8/17 and repetitive behaviors in autism. *Am J Psychiatry* 1999; 156:317-320

18. Ganguly NK, Anand IS, Koicha M, Jindal S, Wahi PL: Frequency of D8/17 B lymphocyte alloantigen in north Indian patients with rheumatic heart disease. *Immunol Cell Biol* 1992; 70 (Pt 1):9-14

19. Kaur S, Kumar D, Grover A, Khanduja KL, Kaplan EL, Gray ED, Ganguly NK: Ethnic differences in expression of susceptibility marker(s) in rheumatic fever/rheumatic heart disease patients. *Int J Cardiol* 1998; 64:9-14

20. Kiessling LS, Marcotte AC, Culpepper L: Antineuronal antibodies: tics and obsessive-compulsive symptoms. *J Dev Behav Pediatr* 1994; 15:421-425

21. Singer HS, Giuliano JD, Hansen BH, Hallett JJ, Laurino JP, Benson M, Kiessling LS: Antibodies against human putamen in children with Tourette syndrome. *Neurology* 1998; 50:1618-1624

22. Laurino JP, Hallett J, Kiessling LS, Benson M, Pelletier T, Kuhn C: An immunoassay for anti-neuronal antibodies associated with involuntary repetitive movement disorders. *Ann Clin Lab Sci* 1997; 27:230-235

Chapter 4

Elevated binding of D8/17-specific antibody to B lymphocytes in tic disorder patients may be due to increased expression of receptors for the constant parts of IgM molecules

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To the editor

Several groups have independently reported elevated D8/17 expression on B lymphocytes in patients with a tic or related disorder, making this a promising peripheral blood marker for these conditions.[1] However, concerns regarding insufficient sensitivity of the D8/17 assay have recently been raised.[2] This prompted us to reanalyze the data of our published study on D8/17 B cell expression in tic disorder patients compared with healthy subjects.[1] Our study was the only study reported so far that used both flow cytometry and a comparison immunoglobulin M (IgM) monoclonal antibody. For this purpose, we used MOC32, an IgM monoclonal antibody that is directed against a neuroendocrine antigen of epithelial origin of small cell lung cancer cells. In contrast to previous studies, we did not assess a percentage of D8/17-positive B cells, since our flow cytometric analysis did not indicate a separate subpopulation of D8/17-positive B cells. Instead, we calculated D8/17 B cell overexpression, by subtracting the mean fluorescence intensity (MFI) produced by MOC32 from the MFI produced by the D8/17-specific monoclonal antibody. At reanalysis of our published data, there appeared to be an unexpected, close correlationship between the MFI produced by the irrelevant IgM, MOC32, and that by the D8/17-specific IgM, both in the 33 tic disorder patients (Pearson's $r=0.730$, $df=31$, $p<0.001$), the 20 healthy comparison subjects ($r=0.839$, $df=18$, $p<0.001$), and the group as a whole ($r=0.753$, $df=51$, $p<0.001$). Also, the median MFI produced by MOC32 appeared to be significantly (Mann-Whitney $U=125.0$, $df=51$, $p<0.001$) higher in the 33 tic disorder patients (median=13.3 arbitrary units) than in the 20 healthy controls (median=8.9), as was the case with the median MFI produced by the D8/17-specific antibody (median in patients=23.9, median in controls=13.4, Mann-Whitney $U=89.5$, $df=51$, $p<0.001$). These results could suggest that, at least in part, we did not detect D8/17 overexpression on B cells in tic disorder patients compared to healthy controls, but rather, increased expression of receptors for the constant parts of IgM molecules (Fc-μ) on B cells, so explaining increased binding of both the D8/17-specific monoclonal antibody, and the irrelevant monoclonal antibody (MOC32). This may be due to a more general state of immune activation. Thus, these results may suggest that tic disorder patients do not express a specific, possibly genetic, susceptibility marker for experiencing autoimmune sequelae in the aftermath of streptococcal infections, but at best show evidence of increased immune activity. Perhaps, previous positive reports were due to a nonspecific increase of the number of Fc-μ receptors on B cells, a possibility that certainly deserves further study.

References

1. Hoekstra PJ, Bijzet J, Limburg PC, Steenhuis MP, Troost PW, Oosterhoff MD, Kord J. Kallenberg CG, Minderaa RB: Elevated D8/17 expression on B Lymphocytes, a marker of rheumatic fever, measured with flow cytometry in tic disorder patients. *Am J Psychiatry* 2001; 158:605-610

2. Hamilton CS, Garvey MA, Swedo SE: Sensitivity of the D8/17 assay. *Am J Psychiatry* 2003; 160:1193-1194

Chapter 5

Elevation of D8/17-positive B lymphocytes in only a minority of Dutch patients with post-streptococcal reactive arthritis (PSRA): a pilot study

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To the editor

The last decennium has witnessed a resurgence of reactive arthritis secondary to infection with beta-hemolytic group A streptococci (GAS).[1] Nowadays, post-streptococcal reactive arthritis (PSRA) is recognized as a clinical syndrome distinct from the classic acute rheumatic fever (ARF).[2–5] The major differences between ARF and PSRA are the predominant age of occurrence and the relative risk of developing carditis. Genetic studies reveal differences in the association of HLA DRB1*01 and HLA DRB1*16 with ARF versus PSRA.[6] These HLA alleles may represent an individual's genetic susceptibility factor for developing a humoral hyper-responsiveness secondary to GAS: the elevated D8/17 binding to B lymphocytes *in vitro* occurs with a high frequency (63%–100%) in ARF and has therefore been proposed as a susceptibility marker for ARF.[7–10] Here we report preliminary data on the binding of monoclonal antibody D8/17 to B lymphocytes in a series of Dutch PSRA patients.

We performed a systematic prospective observational study of eight consecutive patients at a Dutch outpatient department of rheumatology who presented with arthritis after streptococcal pharyngitis in the Dutch region of Friesland between May 1998 and May 1999. In all patients, antistreptolysine O (ASO) and antideoxyribonuclease B (antiDNAse B) titers were measured simultaneously and monitored sequentially at presentation, and 6 weeks and 3 and 6 months after the primary throat infection. A significant rise and fall of ASO and/or antiDNAse B titers was required prior to inclusion, as described previously.[5] Patients were included only if PSRA was diagnosed according to accepted criteria.[1–5]

All PSRA patients were assessed for B cell expression of D8/17, except one whose blood sample was lost. Blood was collected in acid citrate dextran tubes (ACD solution B tubes; Terumo Europe, Leuven, Belgium) and the whole-blood staining procedure was done the same day. Fluorescence-activated cell sorting (FACS) was performed within 24 h. Staining was done by adding 30 µl of immunoglobulin (Ig) M monoclonal antibody (tube A) or 30 µl of the D8/17 antibody (a generous gift from Dr. J.B. Zabriskie, The Rockefeller University, New York, USA) (tube B) to 100 µl of whole blood. After incubation for 1 hour at 4° C, the suspension was washed with 2 ml phosphate-buffered saline (PBS) with 0.5% bovine serum albumin (Sigma Aldrich, Zwijndrecht, the Netherlands) and centrifuged at 2500 rpm for 2 minutes. To both pellets, 5 µl CD19-PE (IQP, Groningen, the Netherlands) and 5 µl goat-anti-mouse-IgM-FITC (Southern Biotechnology Associates, Birmingham, AL, USA) bovine serum albumin (Sigma Aldrich, Zwijndrecht, the Netherlands) were added for half an hour at room temperature. After incubation, the red cells were lysed with 2 ml FACS lysing solution (Becton Dickinson, Leiden, The Netherlands) for 10 minutes, centrifuged, and washed. The pellet was resuspended in 100 µl PBS with 0.5% bovine serum albumin and stored at 4° C until measured on the FACSstar (Woerden, the Netherlands). Measuring was done by placing a gate round the

CD19-positive B cells; 2 000 cells were counted. The results for D8/17-positive B cells obtained by FACS analysis were classified as negative when expression was <8.0% (<P95 as determined in a control group) and as positive when expression was >8.0% (>P95 D8/17).

Eight Dutch patients [female/male ratio 7/1; mean (S.D.) age 32 (12) yr] with arthritis were included (complete data sets for seven patients are shown in Table 1). A positive throat culture with GAS was obtained in only three patients. Arthritis was present in all patients; the mean (S.E.M.) number of affected joints was 7.9 (2.6). No manifestations of carditis, conduction block, or erythema were observed. Transient cholestatic hepatitis was found in one and uveitis in two patients. Prophylaxis by monthly treatment with penicillin was advised for all patients in whom a primary GAS infection was suspected. All patients showed full recovery within a 1-year follow-up period, which was uneventful. The binding of monoclonal antibody D8/17 to B lymphocytes was assessed. The percentage of D8/17-positive B lymphocytes in PSRA patients ranged from 2.1% to 9.6% with a mean (S.D.) 5.5% (2.7%). A control group of 22 unselected (eight females, 14 males) healthy volunteers was used to determine the normal expression of D8/17. The percentage of D8/17-positive B cells had a normal distribution, with range 1.0%–8.0% and mean (S.D.) 4.0% (2.2%). Two of seven PSRA patients (patients 6 and 7) had elevated expression of D8/17 (>8.0%).

We conclude that arthritis secondary to streptococcal infection in our region of the Netherlands is not accompanied by cardiac or neuropsychiatric involvement. Only 29% of PSRA patients had an elevated percentage of D8/17-positive B lymphocytes, which is in contrast with the 63%–100% in the ARF literature. The fact that five of seven (71%) of our patients had a normal percentage of D8/17-positive B lymphocytes may suggest non-susceptibility to developing ARF in the majority of Dutch PSRA patients. Further prospective multicenter studies are warranted to confirm these findings in larger patient populations.

Table 1. *Demographic and laboratory data of seven PSRA patients*

Patient	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*Group data:* **Mean (SD)**
Age (years)	54	18	32	32	31	19	37	32 (12)
Sex	F	F	F	M	F	F	F	F/M=6/1
Previous ARF	-	-	-	-	-	-	-	
Throat culture	GAS	GAS[1]	Neg	Neg	Neg	GAS	Neg	3/7 GAS
Number of arthritic joints	19	6	1	2	3	10	14	8 (7)
Fever	+	-	-	+	+	+	-	+/-=4/3
ESR (mm/hr)	73	13	22	84	59	90	69	59 (30)
CRP (mg/l)	44	3	9	96	40	69	68	47 (34)
D8/17 (%)	2.1	3.2	4.1	4.4	6.9	8.0	9.6	5.5 (2.7)
ASO (U/l)	1200	340	1200	1200	300	2400	300	990 (760)
AntiDNAse B (U/l)	2500	240	1600	500	400	6400	2000	1950 (2150)
ASO/AntiDNAse B ratio	0.48	1.42	0.75	2.40	0.75	0.38	0.15	0.90 (0.77)
Recovery (months)	6	2	3	4	4	2	5	3.7 (1.5)
Extra-articular phenomena	*			**			***	

PSRA=post-streptococcal-reactive arthritis; ARF=acute rheumatic fever; ESR=erythrocyte sedimentation rate; CRP=C-reactive protein; ASO=antistreptolysine O; AntiDNAse B=antideoxyribonuclease B; M=male; F=female; Neg=negative; GAS=group A streptococci; [1]also positive for Haemophilus influenzae

Extra-articular manifestations: *cholestatic hepatitis; **transient nodal escape, minor valve insufficiency and uveitis; ***corticosteroid (topical)-responsive iritis and episcleritis

References

1. Jansen TLTA, Janssen M, Riel PLCM van. Grand rounds in rheumatology: acute rheumatic fever or poststreptococcal reactive arthritis: a clinical problem revisited. *Br J Rheumatol* 1998; 37:335–40.
2. Arnold MH, Tyndall A. Poststrepto-coccal reactive arthritis. *Ann Rheum Dis* 1989;48:686–8.
3. Deighton C. Beta-haemolytic streptococci and reactive arthritis in adults. *Ann Rheum Dis 1993;52:475–82.*
4. Jansen TLTA, Janssen M, Jong AJL de, Jeurissen MEC. Poststreptococcal reactive arthritis: a clinical and serological description, revealing its distinction from acute rheumatic fever. *J Intern Med* 1999;245:261–7.
5. Jansen TLTA, Janssen M, Traksel R, Jong AJL de. A clinical and serological comparison of group A versus non-group A streptococcal reactive arthritis and throat culture negative cases of post-streptococcal reactive arthritis. *Ann Rheum Dis* 1999; 58:410–4.
6. Ahmed S, Ayoub EM, Scornik JC, Wang CY, She JX. Poststreptococcal reactive arthritis. Clinical characteristics and association with HLA-DR alleles. *Arthritis Rheum* 1998; 41:1096–102.
7. Zabriskie JB, Lavenchy D, Williams RC Jr et al. Rheumatic fever-associated B cell alloantigens as identified by monoclonal antibodies. *Arthritis Rheum* 1985;28:1047–51.
8. Taneja V, Mehra NK, Reddy KS et al. HLA-DRuDQ antigens and reactivity to B cell alloantigen D8/17 in Indian patients with rheumatic heart disease. *Circulation* 1989;80:335–40.
9. Ganguly NK, Anand IS, Koicha M, Jindal S, Wahl PL. Frequency of D8/17 B lymphocyte alloantigen in North Indian patients with rheumatic heart disease. *Immunol Cell Biol* 1992;70:9–14.
10. Kaur S, Kumar D, Grover A et al. Ethnic differences in expression of susceptibility marker(s) in rheumatic feverurheumatic heart disease patients*. Int J Cardiol* 1998;64:9–14.

Chapter 6

Increased seroreactivity in tic disorder patients to a 60 kD protein band from a neuronal cell line

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Abstract

Objective: *In tic disorder patients, previous studies have demonstrated increased seroreactivity against crude neuronal antigen extracts. However, no molecular characterization of the antigenic structures involved is available. The aim of this study was to identify and characterize possibly involved target autoantigens.*

Methods: *Eighty-two patients with a tic disorder were compared with 43 healthy control subjects, 15 patients with autistic disorder (AD), and 25 persons with obsessive-compulsive disorder (OCD). In these subjects, seroreactivity against a protein extract from HTB-10 neuroblastoma cells was analyzed by using Western blot techniques. The most relevant identified antigenic structure was subsequently isolated, and subjected to amino acid sequencing.*

Results: *All subjects showed reactivity against a multitude of protein bands. Binding to a protein with a molecular weight of 60 kD occurred significantly more frequently in patients with a tic disorder (67.1%), than in patients with AD (40.0%), OCD patients (40.0%), and healthy controls (41.9%). Sequence analysis identified this protein as a human 60 kD heat shock protein (hsp60). However, the involvement of other autoantigens with a molecular weight of 60 kD cannot be excluded.*

Conclusion: *Hsp60, an antigenic structure that is not exclusive to neuronal tissue, may be an important target auto antigen in tic disorders. These data lend further support to the involvement of autoimmunity in tic disorders.*

Introduction

Over the past decade, several research reports (recently reviewed by Hoekstra et al.[1]) have appeared, which suggested the presence of autoantibodies directed against components of the central nervous system in the serum of patients with tic and related disorders. This may indicate the involvement of autoimmunity in these disorders. The studies involved used a crude antigen substrate, stemming from a neuroblastoma cell line or human or animal brain tissue for detecting these autoantibodies. By means of either immunofluorescence or enzyme-linked immunosorbent assay (ELISA), differences in the presence or levels of antineuronal binding between tic disorder patients and normal controls have, thus, repeatedly been reported. Relatively high levels of antineuronal antibodies, however, have also been found in sera of healthy control subjects. This raises doubts about the exclusive neuronal nature of the epitopes concerned. In general, a major disadvantage of using crude antigen extracts is the possible involvement of a multitude of different antigens, which may include binding sites that are not specific for neuronal tissue.

Only a limited number of studies so far, were directed at identifying separate neuronal antigens, by using Western blot techniques. Most of these studies detected candidate antigenic structures, derived from either brain tissue, or a neuroblastoma cell line, with an apparent molecular weight of 60 and/or 83 kD.[2-8] Typically, in such studies, significantly more patients with a tic disorder than healthy comparison subjects were found to show seroreactivity against proteins of these molecular weights. Until now, molecular characterization of these antigenic structures has not been carried out, however.

In the present study, we intended to further elucidate the antigenic nature of antineuronal antibodies in patients with a tic disorder, by using the immunoblot technique to identify neuronal antigens (originating from the HTB-10 cell line, a human neuroblastoma cell line) recognized by autoantibodies present in sera of patients with a tic disorder, and by sequencing the most relevant antigenic structure(s). Levels of autoantibodies against this HTB-10 cell line had previously been shown to differentiate between patients with Tourette's syndrome and healthy control subjects in an ELISA assay.[9]

Methods

Subjects

Patients with a tic disorder were compared with three control groups: healthy volunteers, and two disease control groups, that is, patients with autistic disorder (AD) and patients with obsessive-compulsive disorder (OCD). Patients with a tic disorder were required to fulfill the criteria for a definite tic disorder according to the Tourette's Syndrome Research Criteria.[10] These criteria require observable tics to be present during the clinical interview to allow for study entry. Patients with AD were diagnosed in accordance with both the Autism Diagnostic Interview-Revised[11] and the Autism Diagnostic Observation Schedule-Generic criteria.[12]

Participants with a diagnosis of OCD had to meet DSM-IV criteria for OCD. Healthy volunteers could only participate in the study when they had never been in contact with mental health services.

Excluded from the study were subjects with a history or family history of an autoimmune disorder. Another exclusion criterion for the comparison groups, including the patients with OCD, was a history or family history of tics. None of the subjects who were willing to participate in the study fulfilled any of these exclusion criteria.

The tic disorder patients and patients with OCD were recruited from the outpatient clinic of the Child and Adolescent Psychiatry Center or from patients' associations, whereas subjects with AD previously participated in other studies at our center. The healthy volunteers were recruited from hospital staff or their children. The aim and procedure of the study were fully explained to the subjects before written consent was requested. If the subjects were under 18, the parents were informed as well, and the written informed consent of the parents and the subject's assent were obtained.

To assess tic severity, we used the Yale Global Tic Severity Scale,[13] which consists of separate scales for motor and vocal tics complemented with a separate rating of impairment. Blood was sampled from the subjects within a week of the psychiatric screening.

Eighty-two tic disorder patients entered the study (55 male, 27 female), ranging in age from 6 to 63 years (mean=22.0, SD=15.4). These were not preselected according to an association of symptom exacerbations with streptococcal infections. Twenty of the 82 tic disorder patients demonstrated clinically relevant obsessions and compulsions comorbid with the tics. However, none of the tic disorder patients met DSM-IV criteria for AD or Asperger's disorder. In addition, a total number of 15 patients with AD (14 boys, one girl) participated in the study, with an age range from 8 to 16 years (mean=11.9, SD=2.7). The OCD comparison group consisted of 25 subjects (eight men, 17 women) and ranged in age from 10 to 54 years (mean=30.3, SD=11.3). Finally, 43 healthy volunteers (26 male, 17 female) entered the study, ranging in age from 5 to 67 years (mean=22.3, SD=16.9). Though there was no significant age difference between the tic disorder patients and the healthy comparison subjects (t-test), the age of the tic disorder patients was significantly higher (t-test=2.5, df=95, p=0.014) than that of the patients with AD, and significantly lower (t-test=-2.5, df=105, p=0.014) than that of the OCD patients.

The mean total Yale Global Tic Severity Scale score for the tic disorder patients was 44.3 (SD=24.2, range=5-100), with a mean motor score of 13.8 (SD=4.9, range=4-25), a mean vocal score of 8.7 (SD=6.4, range=0-25), and a mean impairment score of 22.0 (SD=16.4, range=0-50). Thirty-six of the tic disorder patients were taking psychotropic medication, that is, various antipsychotic agents (N=25), clonidine (N=7), or antidepressive medication (N=4); 46 tic disorder patients were free of medication.

Culture of cell lines and isolation of cell protein extracts

The human neuroblastoma cell line HTB-10 (American Type Culture Collection, Rockville, MD) was used for our experiments, with the larynx carcinoma cell line Hep2 (American Type Culture Collection) serving as non-neuronal control cell line. Cells were grown at 37° C in a 5% CO_2 incubator, in tissue culture flasks containing RPMI 1640 with 25 mM hepes and L-glutamine (Bio-Whittaker, Europe) and 10% fetal calf serum, supplemented with 200 mM glutamine, 100 mM sodium pyruvate, 0.05 M Eagle's Basal Medium, 10 μg/ml gentamycine, and 2.12 μg/ml amphotericin B.

The cell lines were rinsed with Dulbecco's Minimal Essential Medium (DMEM) containing 4.5 g/l glucose and L-glutamine, then trypsinized with DMEM containing 2 mM ethylene glycol-bis-(beta-aminoethyl ether)-N,N,N',N'-tetracetic acid and 2 mM ethylenediamine tetraacetic acid, and subsequently homogenized in 10 mM tris-HCl with 1.5 mM $MgCl_2$, 10 mM NaCl and a cocktail of protease inhibitors to a concentration of 20 000 cells per ml. The homogenate was centrifuged at 800 rpm for 5 minutes. The supernatant was centrifuged at 12 000 rpm for 20 minutes. The resultant pellet was resuspended in 10 mM Tris isolation buffer and stored at –80° C until use.

Western blot

A total of 0.36 mg of each protein extract was subjected to electrophoresis in 7.5% acrylamide gels (Biorad) and then transferred to nitrocellulose. The nitrocellulose was blocked for 1.5 hours with phosphate-buffered saline containing 4% milk and then exposed to serum (diluted 1:250 in 1% milk and 0.1% Tween 20) for 1 hour at room temperature. Sera were kept frozen at –80° C for a maximum period of 3 years prior to analysis. The nitrocellulose was washed and then exposed to the secondary antibody, horseradish peroxidase-conjugated rabbit anti-human immunoglobulin G (IgG) (Dako P214), diluted 1:3500, for 1 hour at room temperature. After washing with phosphate-buffered saline, the bound antibodies were visualized by using enhanced chemiluminescence reagents (Roche). Estimation of molecular weights of the bands was based on the distance migrated for molecular weight standards. This was performed by an examiner (G.H.) who was blind to subject status.

Purification and N-terminal protein sequence analysis of a 60 kD protein

In order to isolate and purify the protein band of a molecular weight of 60 kD, to which most frequent antibody binding occurred in tic disorder patients, we subjected the neuroblastoma protein extract to 7.5% sodium dodecylsulphate-polyacrylamide gel electrophoresis. The gel was then stained with 0.1% Coomassie Brilliant Blue (CBB-R250) in 45% methanol and 10% acetic acid for 30 minutes at room temperature, and destained with 45% methanol and 10% acetic acid. The 60 kD protein band was cut from the gel and subjected to sequence analysis, by performing phenylisothiocyanate degradation. A total number of 12 N-terminal amino acid cycles was determined.

Statistical analysis

Pairwise comparisons between tic disorder patients and the three control groups were carried out by using chi-square analysis, with the dichotomous variable of presence or absence of reactivity against the 60 kD protein. To rule out possible medication and sex effects, we compared tic disorder patients with and without medication, as well as male and female patients by means of chi-square analysis. We used the t-test to investigate possible differences between tic disorder patients with and without seroreactivity against the 60 kD protein, regarding age, and severity of tics as measured by the motor, phonic, and total score on the Yale Global Tic Severity Scale. Also, in each of the three comparison groups, the t-test was used to test the significance of possible age differences between subjects with and without seroreactivity against the 60 kD protein. Given the large range in age between and within subject groups, we decided to also separately perform these analyses for subjects below 17 and subjects of 17 years and older. Finally, group differences were analyzed separately for children below 13, using chi-square. All tests of significance used the 0.05 level of significance and were two-tailed.

Results

In Western blots, IgG in serum from both tic disorder patients and comparison subjects reacted with a multitude of protein bands in the neuroblastoma cell extract. By far the most frequently observed antibody-antigen interaction occurred at a molecular weight of 60 kD. When using an antigen extract from the HEP2 cell line, only very weak reactions against a protein band of 60 kD were detected. Figure 1A shows some representative Western blots, demonstrating presence or absence of seroreactivity against the 60 kD protein when using the neuroblastoma cell line protein extract. Figure 1B shows binding of the same sera against the HEP2 extract. In total, when using the HTB-10 cell line, binding of serum IgG to the 60 kD band was detected in 55 of 82 (that is, 67.1%) patients with a tic disorder, as opposed to 10 of 25 (40.0%) OCD patients, six of 15 (40.0%) patients with AD, and 18 of 43 (41.9%) healthy comparison subjects. Table 1 contains the binding percentages across the subject groups as a whole as well as separately for subjects below and above 17 years, and for children below 13. Regarding the whole group, the percentage of tic disorder patients who showed reactivity against the 60 kD protein was significantly higher than this percentage in healthy comparison subjects (chi-square=7.4, df=1, p=0.007), patients with OCD (chi-square=5.9, df=1, p=0.015), and patients with AD (chi-square=4.0, df=1, p=0.046). Also, when confining the comparisons to subjects below 17, the percentage of tic disorder patients who showed reactivity against the 60 kD protein was significantly higher than this percentage in healthy comparison subjects (chi-square=5.8, df=1, p=0.016) and in patients with AD (chi-square=6.0, df=1, p=0.015). In this age group, no comparisons with OCD patients could be carried out, since only two OCD patients were below 17. Table 1 shows that group differences in binding percentages were similar in magnitude

for the subjects above 17. However, these differences did not reach statistical significance within this age group (chi-square analysis). Differences within children below 13 between tic disorder patients and healthy controls (chi-square=9.1, df=1, p=0.003), and between tic disorder patients and patients with AD (chi-square=7.0, df=1, p=0.008) were both significant. Comparisons of presence or absence of 60 kD seroreactivity between male and female tic disorder patients, or between tic disorder patients with and without medication did not reveal any significant differences, neither in the group as a whole, nor in patients below or above 17 separately. Also, no significant differences were found between tic disorder patients with and without seroreactivity against the 60 kD protein regarding severity of tics as measured by the motor, phonic, and total scores on the Yale Global Tic Severity Scale, neither in the group as a whole, nor in patients below or above 17 separately. However, within the group as a whole, the mean age of tic disorder patients with seroreactivity against the 60 kD protein (mean=18.4 years) turned out to be significantly (t-test=-3.1, df=80, p=0.002) lower than the age of tic disorder patients without this seroreactivity (mean=29.2 years). Separate subgroup analyses for tic disorder patients below and above 17 with regard to anti-60 kD reactivity related to age, only identified significant age differences within the older subject group (mean age of 60 kD reacting tic disorder patients within subjects above 17=32.3; mean age of 60 kD negative subjects within this age group=44.4; t-test=-2.6; df=30; p=0.013), which was not observed in the group of patients below 17. No age differences were found between subjects with and without seroreactivity against the 60 kD protein in the three comparison groups, neither in the group as a whole, nor in patients below or above 17 separately.

Sequence analysis of the aminoterminal region of the isolated 60 kD protein yielded aminoacid sequences (Ala-Lys-Asp-Val-Lys-Phe-Gly-Ala-Asp-Ala-Arg-Ala) that were 100% identical to the N-terminal sequence of the mature chain of a human 60 kD heat shock protein (hsp60). This sequence applied to more than 90% of proteins present that did not show N-terminal blocking.

Table 1. *Percentages of seroreactivity against a protein band with a molecular weight of 60 kD as detected by Western blot analysis using HTB-10 in the group as a whole, as well as in patients categorized according to age.*

	*Whole group**	*Subjects<13***	*Subjects<17***	*Subjects>16****
Tic disorder	67.1% (55/82)	80.8% (21/26)	74.0% (37/50)	56.3% (18/32)
AD	40.0% (6/15)	33.3% (3/9)	40.0% (6/15)	
OCD	40.0% (10/25)	50.0% (1/2)	50.0% (1/2)	39.1% (9/23)
Healthy controls	41.9% (18/43)	35.3% (6/17)	46.2% (12/26)	35.3% (6/17)

AD=autistic disorder; OCD=obsessive-compulsive disorder
*Differences between tic disorder patients and AD, OCD as well as healthy controls are significant.
**Differences between tic disorder patients and AD as well as healthy controls are ignificant.
***Differences between tic disorder patients and OCD as well as healthy controls do not reach statistical significance.

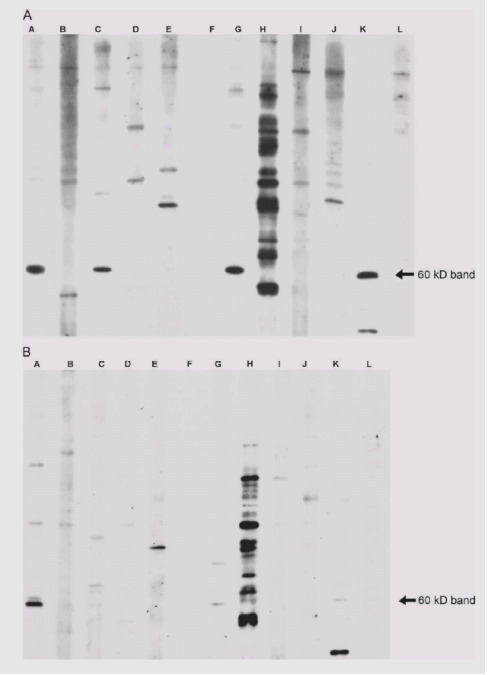

Figure 1A. *Representative Western blots showing sera that demonstrate reaction against hsp60 (lanes A, C, G and K), and other sera that do not show anti-hsp60 binding (lanes B, D, E, H, I, L), when using a protein extract from HTB-10. Lane F does not contain serum. Apart from present or absent reaction against hsp60, binding against several other protein bands is clearly visible. Especially lane H shows many immunoreactive proteins, with yet absent hsp60 binding.*

Figure 1B. *Autoantibody binding patterns of the same sera against the HEP2 protein extract, demonstrating much weaker anti-hsp60 binding.*

Discussion

This immunoblot study showed that autoantibodies present in serum of tic disorder patients do not recognize a single major neuronal antigenic structure. Rather, in each individual patient, binding occurs to a multitude of proteins. Given this finding, previous studies[3,4,9,14,15] which reported higher than usual levels of antineuronal antibodies as measured by ELISA assay or immunofluorescence, should be viewed with caution. In fact, the studies involved simultaneously measured a plethora of antigen antibody interactions.

Still, our study confirms earlier reports[2-4,7] about the relatively frequent involvement of a target autoantigen with a molecular weight of 60 kD, present in neuronal cells. Furthermore, reactivity to human basal ganglia antigens of the same molecular weight have occurred in poststreptococcal acute disseminated encephalomyelitis[16] and Sydenham's chorea.[17] However, an earlier preliminary claim of homology of a 60 kD neuronal antigenic structure, apparently involved in tic disorders, with calpastatin,[6] could not be confirmed by the results of our sequence analysis. Instead, we identified the 60 kD protein as a human heat shock protein.

Western blot analyses aim to identify possibly relevant antigenic structures and do not quantify antibody levels. Antibodies to hsp60 are known to be present in virtually all individuals.[18] Apparently, our immunoblot assay only detects anti-hsp60 binding above a certain threshold level. Also, the HTB-10 cell line may

theoretically contain more than one 60 kD protein, especially as far as such a protein would show N-terminal blocking, which would not make it susceptible to the sequence analysis. Reactions against such additional 60 kD proteins would not be distinguishable from anti-hsp60 bindings on Western blots. Thus, though our results strongly suggest elevated serum anti-hsp60 titers in tic disorder patients, specific anti-hsp60 ELISA assays are needed to quantify this autoantibody. Also, a 2-D gel electrophoresis may be used in future studies, to demonstrate whether indeed only a single 60 kD protein would be involved. In addition, it should be stressed that the presence of human hsp60 is by no means confined to neuronal tissue. Indeed, hsp60 is ubiquitously present. It is found intracellularly, and functions as a molecular chaperone protein involved in the assembly and folding of proteins.[19] In addition, hsp60 can be released into the extracellular environment, and has been identified in the peripheral circulation of normal individuals.[20] Neuronal cells, including neuroblastoma cell lines, have specifically been demonstrated to secrete hsp60 in response to toxic agents.[21] Hsp60 is thus thought to serve also extracellularly a protective role, intended to prevent cellular damage. Protection against cell death is of critical importance to neuronal cells, given their lack of cell-dividing capabilities. Differences between anti-hsp60 reactivity when using the neuronal cell line versus when using the Hep2 cell line may be explained against the background of a more profound role and, possibly, elevated quantity of hsp60 within neuronal tissue.

There is accumulating evidence that inappropriate reactivity to heat shock proteins is involved in autoimmune disorders.[22] Abnormal reactivity to hsp60 has been most notably linked to rheumatic autoimmune diseases.[23-25] For instance, increased serum levels of antibodies to hsp60 have been found in children with juvenile chronic arthritis.[26] Also, the association between elevated anti-hsp60 levels and severe coronary heart disease and carotid atherosclerosis is well-established.[27]

Given the high degree of antigenic homology between microbial (bacterial and parasitic) and human hsp60, immunity to microbes may lead to harmful cross-reactivity with human structures.[28] Also, in patients with tic and related disorders, an association of infections with symptom exacerbations has been suggested.[29] Apart from induction through bacterial infections, genetic factors may well be involved in increased anti-hsp60 antibody levels: a strong association between IL-6-174 gene polymorphism and anti-hsp60 titers has recently been described.[30] This fits remarkably well to the relevance of both genetic factors and environmental factors in tic disorders. Thus, tic disorder patients with elevated anti-hsp60 titers may well form a relatively homogeneous subgroup, useful for the design of genetic studies.

Though we used a neuroblastoma cell line as antigenic substrate in our experiment, it remains to be investigated whether any reactivity occurs at all against hsp60 in neuronal tissue *in vivo*. One finding which favors true antibody-antigen interactions in the central nervous system, relevant to the pathogenesis of tic disorders, comes from a recently developed animal model[31,32] in which the transfer of antineuronal antibodies of children with Tourette's syndrome to the

striatum of rats induced stereotypic movements and utterances in these animals. Future studies should investigate the pathophysiologic relevance, if any, of anti-hsp60 interactions in the central nervous system.

An interesting feature of antibodies to hsp60 is their reported cross-reactivity to pathogenetically relevant autoantigens in several disease states, including Lyme's disease[33] and rheumatoid arthritis.[34] Thus, it may well be that our finding of more frequent binding to hsp60 in tic disorder patients actually reflects increased antibody binding to a disease-specific epitope that remains to be identified.

In our study, increased detection of anti-hsp60 was confined to tic disorder patients. The patients with AD and the OCD patients showed anti-hsp60 reactivity that was remarkably similar to the healthy comparison subjects. Still, future studies that examine anti-hsp60 antibody levels in tic disorder patients, may consider to include patients with schizophrenia, given some reports of the latter patient's increased anti-hsp60 seroreactivity.[35-38] Other studies may want to investigate the role of age, in combination with the duration of illness. While we failed to systematically collect data regarding disease duration in the present study, we did find an association between lower tic disorder patients' age and seroreactivity against hsp60. This was largely due to the lower mean age of adult patients showing anti-hsp60 binding. We did not observe this age difference in the pediatric patients, which makes this finding hard to interpret. The appearance of the tics may have been induced by infections that are thought to evoke antibodies that cross-react with human tissue. This might explain the apparently increased levels of anti-hsp60 autoantibodies in younger patients, given these patients' shorter time frame since the first occurrence of the tics. Possibly, children's general tendency to show increased anti-hsp antibodies due to their heightened frequency of infections, may initially obscure the disease duration effect. In this respect, it is of interest to note that tics are also far more common in children than in adults.[39] Longitudinal studies, however, are needed to examine the course and significance of alterations of anti-hsp60 autoantibody levels over time, related to disease development. In addition, it would be of interest to study autoantibody reactions at the onset of the disorder, since later autoantibody repertoires can reflect responses to damaged tissues,[40] and, thus, could be the consequence rather than the cause of the underlying mechanism accounting for the disease process. If, however, anti-hsp60 antibodies would indeed be involved in the etiopathogenesis of tic disorders, such studies should find increased levels of these antibodies early in the course of illness, rather than later.

In conclusion, the present finding of elevated anti-hsp60 reactivity in serum of patients with a tic disorder adds to existing data, which may support the involvement of autoimmunity in tic disorders. We are, however, far from understanding the precise complex interplay between genetic and environmental factors in tic and related neuropsychiatric disorders.

References

1. Hoekstra, P.J., Kallenberg, C.G., Korf, J., Minderaa, R.B. 2002. Is Tourette's syndrome an autoimmune disease? *Mol. Psychiatry* 7, 437-445.
2. Church, A.J., Dale, R.C., Lees, A.J., Giovannoni, G., Robertson, M.M. 2003. Tourette's syndrome: a cross sectional study to examine the PANDAS hypothesis. *J. Neurol. Neurosurg. Psychiatry* 74, 602-607.
3. Singer, H.S., Giuliano, J.D., Hansen, B.H., Hallett, J.J., Laurino, J.P., Benson, M., Kiessling, L.S. 1998. Antibodies against human putamen in children with Tourette syndrome. *Neurology* 50, 1618-1624.
4. Singer, H.S., Giuliano, J.D., Hansen, B.H., Hallett, J.J., Laurino, J.P., Benson, M., Kiessling, L.S. 1999. Antibodies against a neuron-like HTB-10 neuroblastoma cell in children with Tourette syndrome. *Biol. Psychiatry* 46, 775-780.
5. Trifiletti, R.R., Altemus, M., Zabriskie, J. 1998. TS83: Candidate Target Autoantigen in Tourette Syndrome and OCD. *Biol. Psychiatry*. 43, 72S [Abstract].
6. Trifiletti, R.R., Bandele, A.N. 2000a. Antibodies to the Calpain-Calpastatin Complex in Patients with Tics, Tourette Syndrome, or Obsessive-Compulsive Disorder. *Ann. Neurol*. 48, 542 [Abstract].
7. Trifiletti, R.R., Bandele, A.N. 2000b. Serum Antibodies to Specific Brain Proteins in Patients with Tics, Tourette's Syndrome, or Obsessive-Compulsive Disorder. *Ann. Neurol.* 48,511-512 [Abstract].
8. Wendlandt, J.T., Grus, F.H., Hansen, B.H., Singer, H.S. 2001. Striatal antibodies in children with Tourette's syndrome: multivariate discriminant analysis of IgG repertoires. *J. Neuroimmunol*. 119, 106-113.
9. Laurino, J.P., Hallett, J., Kiessling, L.S., Benson, M., Pelletier, T., Kuhn, C. 1997. An immunoassay for anti-neuronal antibodies associated with involuntary repetitive movement disorders. *Ann. Clin. Lab. Sci.* 27, 230-235.
10. Kurlan, R. 1997. Diagnostic criteria for genetic studies of Tourette syndrome. *Arch. Neurol.* 54, 517-518.
11. Lord, C., Rutter, M., Le Couteur, A. 1994. Autism Diagnostic Interview-Revised: a revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. *J. Autism. Dev. Disord.* 24, 659-685.
12. Lord, C., Rutter, M., Goode, S., Heemsbergen, J., Jordan, H., Mawhood, L., Schopler, E. 1989. Autism diagnostic observation schedule: a standardized observation of communicative and social behavior. *J. Autism. Dev. Disord.* 19, 185-212.
13. Leckman, J.F., Riddle, M.A., Hardin, M.T., Ort, S.I., Swartz, K.L., Stevenson, J, Cohe, D.J. 1989. The Yale Global Tic Severity Scale: initial testing of a clinician-rated scale of tic severity. *J. Am. Acad. Child Adolesc. Psychiatry* 28, 566-573.
14. Kiessling, L.S., Marcotte, A.C., Culpepper, L. 1994. Antineuronal antibodies. tics and obsessive-compulsive symptoms. J. Dev. *Behav. Pediatr*. 15, 421-425.
15. Kiessling, L.S., Marcotte, A.C., Culpepper, L. 1993. Antineuronal antibodies in movement disorders. *Pediatrics* 92, 39-43.
16. Dale, R.C., Church, A.J., Cardoso, F., Goddard, E., Cox, T.C., Chog, W.K., Wiliams, A., Klein, N.J., Neville, B.G., Thompson, E.J., Giovannoni, G. 2002. Poststreptococcal acute disseminated encephalomyelitis with basal ganglia involvement and auto-reactive antibasal ganglia antibodies. *Ann. Neurol.* 50, 588-595.
17. Church, A.J., Cardoso, F., Dale, R.C., Lees, A.J., Thompson, E.J., Giovannoni, G. 2002. Anti-basal ganglia antibodies in acute and persistent Sydenham's chorea. *Neurology* 23, 227-231.
18. Prohaszka, Z., Duba, J., Horvath, L., Csaszar, A., Karadi, I., Szebeni, A., Singh, M., Fekete, B., Romics, L., Fust, G. 2001. Comparative study on antibodies to human and bacterial 60 kDa heat shock proteins in a large cohort of patients with coronary heart

disease and healthy subjects. *Eur. J. Clin. Invest*. 31, 285-292.

19. Bukau, B., Horwich, A.L. 1998. The Hsp70 and Hsp60 chaperone machines. *Cell* 92, 351-366.

20. Pockley, A.G., Bulmer, J., Hanks, B.M., Wright, B.H. 1999. Identification of human heat shock protein 60 Hsp60 and anti-Hsp60 antibodies in the peripheral circulation of normal individuals. *Cell. Stress. Chaperones* 4, 29-35.

21. Bassan, M., Zamostiano, R., Giladi, E., Davidson, A., Wollman, Y., Pitman, J., Hauser, J., Brenneman, D. E., Gozes, I. 1998. The identification of secreted heat shock 60 -like protein from rat glial cells and a human neuroblastoma cell line. *Neurosci. Lett.* 250, 37-40.

22. Multhoff, G., Botzler, C., Issels, R. 1998. The role of heat shock proteins in the stimulation of an immune response. *Biol. Chem.* 379, 295-300.

23. MacHt, L.M., Elson, C.J., Kirwan, J.R., Gaston, J.S., Lamont, A.G., Thompson, J.M., Thompson, S.J. 2000. Relationship between disease severity and responses by blood mononuclear cells from patients with rheumatoid arthritis to human heat-shock protein 60. *Immunology* 99, 208-214.

24. Van Roon, J.A., Van Eden, W., Van Roy, J.L., Lafeber, F.J., Bijlsma, J.W. 1997. Stimulation of suppressive T cell responses by human but not bacterial 60-kD heat-shock protein in synovial fluid of patients with rheumatoid arthritis. *J. Clin. Invest*. 100, 459-463.

25. Yokota, S.I., Hirata, D., Minota, S., Higashiyama, T., Kurimoto, M., Yanagi, H., Yura, T., Kubota, H. 2000. Autoantibodies against chaperonin CCT in human sera with rheumatic autoimmune diseases. comparison with antibodies against other Hsp60 family proteins. *Cell. Stress. Chaperones* 5, 337-346.

26. Graeff-Meeder, E.R., Rijkers, G.T., Voorhorst-Ogink, M.M., Kuis, W., van der Zee, R., van Eden, W., Zegers, B.J. 1993. Antibodies to human HSP60 in patients with juvenile chronic arthritis, diabetes mellitus, and cystic fibrosis. *Pediatr. Res*. 34. 424-428.

27. Zhu, J., Quyyumi, A.A., Rott, D., Csako, G., Wu, H., Halcox, J., Epstein, S.E. 2001. Antibodies to human heat-shock protein 60 are associated with the presence and severity of coronary artery disease: evidence for an autoimmune component of atherogenesis. *Circulation* 103, 1071-1075.

28. Wick, G., Perschinka, H., Millonig, G. 2001. Atherosclerosis as an autoimmune disease: an update. *Trends. Immunol.* 22, 665-669.

29. Garvey M.A., Giedd J., Swedo S.E. 1998. PANDAS: the search for environmental triggers of pediatric neuropsychiatric disorders. Lessons from rheumatic fever. *J. Child Neurol.* 13, 413–423.

30. Veres, A., Prohaszka, Z., Kilpinen, S., Singh, M., Fust, G., Hurme, M. 2002. The promoter polymorphism of the IL-6 gene is associated with levels of antibodies to 60-kDa heat-shock proteins. I*mmunogenetics* 53, 851-856.

31. Hallett, J.J., Harling-Berg, C.J., Knopf, P.M., Stopa, E.G., Kiessling, L.S. 2000. Anti-striatal antibodies in Tourette syndrome cause neuronal dysfunction*. J. Neuroimmunol.* 111, 195-202.

32. Taylor, J.R., Morshed, S.A., Parveen, S., Mercadante M.T., Scahill, L., Peterson, B.S., King, R.A., Leckman, L.F., Lombroso, P.J. 2002. An animal model of Tourette's syndrome. *Am. J. Psychiatry* 159, 657-660.

33. Dai, Z., Lackland, H., Stein, S., Li, Q., Radziewicz, R., Williams, S., Sigal, L.H. 1993. Molecular mimicry in Lyme disease: monoclonal antibody H9724 to B. burgdorferi flagellin specifically detects chaperonin-HSP60. *Biochim. Biophys. Acta*. 1181, 97-100.

34. Menge, T., Rzepka, R., Melchers, I. 2002. Monoclonal autoantibodies from patients with autoimmune diseases: specificity, affinity and crossreactivity of Monoclonal antibodys binding to cytoskeletal and nucleolar epitopes, cartilage antigens and mycobacterial heat-shock protein 60. I*mmunobiology* 205.1-16.

35. Schwarz, M.J., Riedel, M., Gruber, R., Ackenheil, M., Muller, N. 1999. Antibodies to heat shock proteins in schizophrenic patients: implications for the mechanism of the disease. *Am. J. Psychiatry* 156, 1103-1104.

36. Mazeh, D., Sirota, P., Patya, M., Novogrodsky, A. 1998. Antibodies to neuroblastoma cell line proteins in patients with schizophrenia. *J. Neuroimmunol.* 84, 218-222.

37. Kilidireas, K., Latov, N., Strauss, D.H., Gorig, A.D., Hashim, G.A., Gorman, J.M., Sadiq, S.A. 1992. Antibodies to the human 60 kDa heat-shock protein in patients with schizophrenia. *Lancet* 340, 569-572.

38. Leykin, I., Spivak, B., Weizman, A., Cohen, I.R., Shinitzky, M. 1999. Elevated cellular immune response to human heat-shock protein-60 in schizophrenic patients. *Eur. Arch. Psychiatry Clin. Neurosci*. 249, 238-246.

39. Burd, L., Kerbeshian, P.J., Barth, A., Klug, M.G., Avery, P.K., Benz, B. Long-term follow-up of an epidemiologically defined cohort of patients with Tourette syndrome. 2001. *J. Child Neurol*. 16, 431-437.

40. Bornstein, N.M., Aronovich B., Korczyn, A.D., Shavit, S., Michaelson, D.M., Chapman, J. 2001. Antibodies to brain antigens following stroke. *Neurology* 56, 529-530.

Chapter 7

Plasma kynurenine and tryptophan in Tourette's and chronic motor tic disorder

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Abstract

Objective: *Increased levels of plasma kynurenine have been reported in tic disorder patients, and this observation has been suggested to be indicative of immune dysregulation in tic disorders. In the present study, we aimed to replicate this finding in a large group of well-characterized tic disorder patients.*

Methods: *Plasma concentrations of tryptophan and kynurenine were determined in Dutch patients with Tourette's disorder (N=44), patients with chronic motor tic disorder (N=15), and healthy volunteers (N=32). Correlations between Yale Global Tic Severity Scale scores and the plasma levels were examined.*

Results: *Our results revealed no significant differences between patients and controls in plasma tryptophan and kynurenine concentrations or in the kynurenine to tryptophan ratio. Also, kynurenine did not correlate with indices of tic severity. A small, but statistically significant negative relationship (r=-0.341; p=0.009) was found between tryptophan levels and vocal, but not motor tic severity. These results indicate that the metabolism of tryptophan through the kynurenine pathway is not altered in tic disorders.*

Conclusion: *Although it is unclear how much may be inferred about immune function based on measurement of plasma kynurenine, the present results do not lend support to hypothesized alterations in immune processes in tic disorder patients.*

Introduction

Tic disorders constitute a spectrum of complex neuropsychiatric disorders characterized by the presence of diverse motor and/or vocal tics, often accompanied by behavioral abnormalities.[1,2] The pathogenesis of tic disorders is not well understood. The possible involvement of immune dysfunction has attracted considerable research attention and has been recently reviewed.[3] In short, studies have pointed to the relevance of overexpression of a B lymphocyte surface marker, designated D8/17,[4] to the possible role of antineuronal autoantibodies,[5] and to an association with antistreptococcal antibodies.[6]

In addition, investigators have reported on elevated levels of the tryptophan metabolite kynurenine in Tourette's disorder (TD) patients.[7,8] This increase in kynurenine has been suggested to be another indication of immune dysregulation in tic disorders. Increased kynurenine levels are a well-known marker of immune activation across a wide range of inflammatory conditions. This can be explained by the effects of pro-inflammatory cytokines, most notably interferon-gamma, on indoleamine 2,3-dioxygenase (IDO), which promotes the breakdown of tryptophan to kynurenine.[9] IDO is present in both brain and non-brain tissue, including lung, kidney and circulating macrophages. Across a wide range of systemic immune activation, it has been found that increases in cerebrospinal fluid levels of kynurenine are substantially greater than increases in plasma levels.[10] Still, plasma kynurenine has also been shown to be increased through immune activation.[11] The kynurenine to tryptophan (K/T) ratio in plasma has been suggested to be a direct measure for IDO activity, and has been found to correlate with neopterin, a well-known marker of cellular immune activation.[12] Finally, subsequent metabolites of the kynurenine pathway are known to be neurotoxic, and may possibly be involved in the pathogenesis of tics or associated symptoms.[13,14] In the present study, we aimed to replicate the finding of elevated plasma kynurenine, and to examine levels of plasma tryptophan in a large series of tic disorder patients.

Methods

Subjects

Two groups of subjects were chosen for the study: healthy volunteers and patients with a tic disorder according to DSM-IV criteria.[15] The tic disorder patients were recruited from the outpatient clinic of the Child and Adolescent Psychiatry Center or from members of the TD patients' association of the Netherlands. The aim and procedures of the study were fully explained to the subjects before written consent was requested. If the subjects were under 18, the parents were informed and the written informed consent of the parents and the subject's assent were obtained.

Thirty-two age-matched healthy volunteers, recruited from hospital staff or their children (17 men, 15 women; 5 to 53 years of age; mean age=18.9, SD=12.4), and 59 tic disorder patients (41 men, 18 women; 6 to 53 years of age;

mean age=17.3, SD=11.8) entered the study. Both study groups were free of chronic illnesses known to affect the immune system. Forty-four of the tic disorder patients met DSM-IV criteria for TD, the remaining 15 tic disorder patients fulfilled DSM-IV criteria for chronic motor tic disorder (CMT). Tic severity was assessed using the motor and vocal scores of the Yale Global Tic Severity Scale.[16] The sum of both scores was used as a measure of total tic severity. The mean total tic severity for the tic disorder patients was 20.5 (SD=9.15, range=5–41), with a mean motor score of 12.7 (SD=4.73, range=4-24), and a mean vocal score of 7.88 (SD=5.99, range=0–22). Table 1 shows data regarding age, sex, and tic severity scores across both groups of tic disorder patients and healthy controls.

Twenty-seven of the patients were taking psychotropic medication, either clonidine (N=5), various antipsychotic agents (N=21), or a selective serotonin reuptake inhibitor (N=1); 32 were free of medication.

Table 1. *Subject characteristics regarding age, sex, and tic severity scores across the two tic disorder groups and the healthy controls.*

	Tourette's disorder	*Chronic motor tic disorder*	*Healthy controls*
	N=44 (35 M, 9 F)	*N*=15 (6 M, 9 F)	*N*=32 (17 M, 15 F)
	Mean (SD), range	**Mean (SD), range**	**Mean (SD), range**
Age	17.1 (11.5), 7-53	17.9 (12.8), 6-46	18.9 (12.4), 5-53
Motor tic score	13.0 (4.61), 4-24	11.7 (5.11), 5-20	
Vocal tic score	10.6 (4.34), 3-22		
Total tic score	23.6 (8.22), 9-41	11.7 (5.11), 5-20	

M=male; F=female

Laboratory measures

Blood was sampled from all subjects during the morning, between 10 AM and noon. This took place within 1 week of each patient's psychiatric screening. All tests were carried out using plasma samples which had been kept frozen at -80° C for a maximum period of 3 years prior to analysis. Plasma tryptophan and kynurenine were determined by high performance liquid chromatography using a modification of the method of Widner et al.[17] Briefly, 100 µl plasma samples were deproteinized with 100 µl of 0.7 M perchloric acid after addition of 50 µl of 25% ascorbic acid and 500 ng of the internal standard, 5-hydroxytryptophan. Supernatants were directly injected on a C_{18} column (mobile phase 96%, pH 3.7, 1.5% acetic acid/4% methanol, 1 ml/min) and measured fluorometrically (tryptophan, 285/345 nm excitation/emission wavelengths) and by ultraviolet absorbance (kynurenine, 360 nm) with within-day and day-to-day coefficients of variation of less than 7%.

Statistical analysis

We used multiple analysis of variance (MANOVA) to test for differences in plasma tryptophan and kynurenine concentrations between patients with TD, patients with CMT, and comparison subjects, as well as between male and female subjects and between patients with and without medication. With analysis of variance (ANOVA), we investigated possible differences between the groups in the K/T ratio, using post-hoc t-tests to separately compare the tic disorder subgroups to the normal control group. In addition, Pearson's correlation test was used to assess the relationship between tryptophan and kynurenine across all subjects, and between the laboratory measures and the total as well as motor and vocal tic scores of the Yale Global Tic Severity Scale across the tic disorder patients as a whole. Also, correlations of the test results with age in tic disorder patients and controls were performed. All tests of significance used the 0.05 level of significance and were two-tailed.

Results

MANOVA found no significant effect of diagnostic group when patients with TD, patients with CMT, and healthy controls were compared with regard to plasma tryptophan and kynurenine ($F=1.60$, $p=0.212$). Also, no effects of medication status ($F=0.346$, $p=0.709$) or gender ($F=0.387$, $p=0.681$) were observed. Table 2 shows mean values of tryptophan, kynurenine, and the K/T ratio across the groups. In a separate ANOVA, no significant diagnostic group effect was seen on the K/T ratio ($F=2.82$, $p=0.065$). Given the trend-level significance seen in the group effect on the K/T ratio, we proceeded to separately compare the tic disorder subgroups to the normal control group using post-hoc t-tests (TD versus normal controls, $t=-1.74$, $p=0.086$; CMT versus normal controls, $t=0.809$, $p=0.423$). No significant correlations were observed between kynurenine and indices of tic severity (eg, $r=0.024$, $p=0.857$ regarding kynurenine versus total tic severity). However, the K/T ratio showed a weak, but statistically significant correlation with vocal ($r=0.330$, $p=0.011$), but not motor ($r=0.150$, $p=0.260$) tic severity. In healthy controls, tryptophan values correlated weakly with kynurenine values ($r=0.372$, $p=0.036$), which we did not find in the tic disorder patients ($r=0.151$, $p=0.254$). Within the tic disorder patients, we found a small, but statistically significant negative correlation between tryptophan concentration and vocal ($r=-0.341$, $p=0.009$) as well as total tic severity ($r=-0.311$, $p=0.017$). In contrast, we found no significant correlationship between tryptophan and motor tic severity ($r=-0.169$, $p=0.204$). Finally, age appeared to be positively correlated with tryptophan concentrations in the healthy controls only ($r=0.484$, $p=0.005$). This relationship between age and tryptophan was not observed in the tic disorder patients ($r=0.080$, $p=0.546$). In addition, across both the healthy and the tic disorder group, age was not significantly correlated with kynurenine ($r=0.135$, $p=0.460$, and $r=-0.113$, $p=0.395$, respectively).

Table 2. *Plasma concentrations of kynurenine and tryptophan, and the mean kynurenine to tryptophan ratio in tic disorder patients and healthy controls. No significant differences were found between the groups in any of the measures.*

	Tourette's disorder	*Chronic motor tic disorder*	*Healthy controls*
	N=44	*N*=15	*N*=32
	Mean (SD)	**Mean (SD)**	**Mean (SD)**
Kynurenine (ng/ml)	409 (106)	377 (117)	379 (88.3)
Tryptophan (µg/ml)	11.3 (2.67)	12.1 (1.91)	11.7 (3.06)
Kynurenine/tryptophan (x1000)	38.1 (12.8)	31.3 (9.13)	33.5 (8.50)

Discussion

Contrary to two previous reports,[7,8] our study failed to find significant differences in plasma kynurenine concentration between tic disorder patients and healthy comparison subjects. Another indication that plasma kynurenine is not associated with tics, was the absence of any tendency for kynurenine levels to correlate with indices of tic severity. Although there was a trend toward a greater K/T ratio in the TD group compared to the control group, this was not encountered at all in the CMT group. In our study, individual kynurenine values varied substantially, and did not correlate with tryptophan within the tic disorder patients. Apart from possible cytokine-induced IDO activation, the activities of the liver tryptophan metabolising enzyme tryptophan 2,3-dioxygenase[18] and the kynurenine metabolizing enzymes kynureninase and kynurenine hydroxylase[19] are known to affect plasma kynurenine levels; variation in these other enzymes may have obscured the effects of IDO activation.

Plasma tryptophan concentrations of the tic disorder patients in the present study were not different from those of the healthy controls. This is in contrast with two independent studies[20,21] that had previously reported slightly, but significantly, decreased serum tryptophan levels in tic disorder patients. However, the results of our study may still support some association of tic disorders with alterations in tryptophan, given the significant negative correlation of tryptophan levels with vocal as well as total tic severity. In addition, tryptophan was not positively correlated with age in the patients, in contrast with the situation in the controls. These two findings are in accordance with previous studies. Comings[20] also found an absent correlation between age and tryptophan in tic disorder patients, whereas Lepage et al.[22] demonstrated a steady increase of tryptophan levels throughout infancy, childhood, and adolescence in healthy controls. Currently, we do not have an explanation for this difference between tic disorder patients and healthy controls.

In conclusion, it appears that in our group of tic disorder patients the metabolism of tryptophan through the kynurenine pathway is not altered. In addition, changes in plasma tryptophan, if present, appear to be quite subtle. Although it is unclear how much may be inferred about immune function based on

measurement of plasma kynurenine, the results do not lend support to hypothesized alterations in immune processes in tic disorders. Thus, while previous studies have identified a number of possible interesting laboratory indicators of immune dysfunctioning in tic disorder patients, it now appears unlikely that the measurement of plasma kynurenine will provide confirming evidence of the possible involvement of autoimmune phenomena in tic disorders.

Given the likelihood that immune mechanisms are operating in at most a subset of tic disorder patients, future studies in this area may benefit from focusing on those patients with abnormal immune indices and on those with markedly elevated plasma kynurenine or K/T ratio.

References

1. Jankovic, J., 2001. Tourette's syndrome. *N. Engl. J. Med*. 345,1184-1192.
2. Leckman, J.F., Peterson, B.S., Anderson, G.M., Arnsten, A.F., Pauls, D.L., Cohen, D.J., 1997. Pathogenesis of Tourette's syndrome. *J. Child Psychol. Psychiatry* 38, 119-142.
3. Hoekstra, P.J., Kallenberg, C.G., Korf, J., Minderaa, R.B., 2002. Is Tourette's syndrome an autoimmune disease? *Mol. Psychiatry* 7, 437-445.
4. Hoekstra, P.J., Bijzet, J., Limburg, P.C., Steenhuis, M.P., Troost, P.W., Oosterhoff, M.D., Korf, J., Kallenberg, C.G., Minderaa, R.B., 2001. Elevated D8/17 expression on B-Lymphocytes, a marker associated with rheumatic fever, in tic disorder patients, measured with flow cytometry. *Am. J. Psychiatry* 158, 605-610.
5. Singer, H.S., Giuliano, J.D., Hansen, B.H., Hallett, J.J., Laurino, J.P., Benson, M., Kiessling, L.S., 1998. Antibodies against human putamen in children with Tourette syndrome. *Neurology* 6, 1618-1624.
6. Muller, N., Kroll, B., Schwarz, M.J., Riedel, M., Straube, A., Lutticken, R., Reinert, R. R., Reineke, T. Kuhnemund, O., 2001. Increased titers of antibodies against streptococcal M12 and M19 proteins in patients with Tourette's syndrome. *Psychiatry Res*. 101, 187-193.
7. Rickards, H., Dursun, S. M., Farrar, G., Betts, T., Corbett, J.A., Handley, S.L., 1996. Increased plasma kynurenine and its relationship to neopterin and tryptophan in Tourette's syndrome. *Psychol. Med*. 26, 857-862.
8. Gaynor, C.M., Rickards, E.H.G., Kariyawasam, S.H., Sidey, F.M., Robertson, M.M., Corbett, J.A., 1997. Increased plasma kynurenine in Tourette syndrome may be due to induction of IDO. *J. Psychopharmacol*. 11, A23.
9. Heyes, M.P., Saito, K., Crowley, J.S., Davis, L.E., Demitrack, M.A., Der, M., Dilling, L.A., Elia, J., Kruesi, M.J., Lackner, A., 1992b. Quinolinic acid and kynurenine pathway metabolism in inflammatory and non-inflammatory neurological disease. *Brain* 115, 1249-1273.
10. Heyes, M.P., Brew, B.J., Saito, K., Quearry, B.J., Price, R.W., Lee, K., Bhalla, R.B., Der, M., Markey, S.P., 1992a. Inter-relationships between quinolinic acid, neuroactive kynurenines, neopterin and β_2-microglobulin in cerebrospinal fluid and serum of HIV-1-infected patients. *J. Neuroimmunol*. 40, 71-80.
11. Maes, M., Verkerk, R., Bonaccorso, S., Ombelet, W., Bosmans, E., Scharpe, S., 2002. Depressive and anxiety symptoms in the early puerperium are related to increased degradation of tryptophan into kynurenine, a phenomenon which is related to immune activation. *Life Sci.* 71, 1837-1848.
12. Widner, B., Laich, A., Sperner-Unterweger, B., Ledochowski, M., Fuchs, D. 2002. Neopterin production, tryptophan degradation, and mental depression-What is the link? *Brain, Behav. Immun*. 16, 590-595.
13. McCreary, A. C., Handley, S. L., 1995. Kynurenine potentiates the DOI headshake in mice. *J. Psychopharmacol.* 9, 68-69.
14. Gaynor, C.M., Handley, S.L., 2001. Effects of nicotine on head-shakes and tryptophan metabolites. *Psychopharmacology (Berl.)* 153, 327-333.
15. American Psychiatric Association, 1994. *Diagnostic and Statistical Manual of Mental Disorders*, 4th ed. American Psychiatric Press, Washington.
16. Leckman, J.F., Riddle, M.A., Hardin, M.T., Ort, S.I., Swartz, K.L., Stevenson, J., Cohen, D.J., 1989. The Yale Global Tic Severity Scale: initial testing of a clinician-rated scale of tic severity. *J. Am. Acad. Child Adolesc. Psychiatry* 28, 566-573.
17. Widner, B., Werner, E.R., Schennach, H., Wachter, H., Fuchs, D., 1997. Simultaneous measurement of serum tryptophan and kynurenine by HPLC. *Clin. Chem*. 43, 2424-2426.
18. Yamazaki, F., Kuroiwa, T., Takikawa, T., Kido, R., 1985. Human indoleamine 2,3-dioxygenase, its tissue distribution, and characterization of the placental enzyme. *Biochem. J*. 230, 635-638.

19. Bender, D.A., Njagi, E.N.M., Danielian, P.S., 1991. Comparison of tryptophan metabolism in vivo and in isolated hepatocytes from vitamin B6 deficient mice. *Adv. Exp. Med. Biol*. 294, 359-368.

20. Comings, D.E., 1990. Blood serotonin and tryptophan in Tourette syndrome. *Am. J. Med. Genetics* 36, 418-430.

21. Leckman, J.F., Anderson, G.M., Cohen, D.J., Ort, S., Harcherik, D.F., Hoder, E.L., Shaywitz, B.A., 1984. Whole blood serotonin and tryptophan levels in Tourette's disorder: effects of acute and chronic clonidine treatment. *Life Sci*. 35, 2497-2503.

22. Lepage, N., McDonald, N., Dallaire, L., Lambert, M., 1997. Age-specific distribution of plasma amino acid concentrations in a healthy pediatric population. *Clin. Chem*. 43, 2397-2402.

Chapter 8

Association of common cold with exacerbations in tic disorder patients: a prospective longitudinal study

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Abstract

Objective: *Cross-sectional data and some case studies suggest a temporal relationship between fluctuations in tic severity and preceding infections. The aim of the present study was to examine this possible relationship in a prospective longitudinal design.*

Methods: *Two groups of tic disorder patients were included in this study, a pediatric group between 7 and 15 years (N=20), and an adult group of 16 years and older (N=41). During a 24 weeks' period, participants were asked to weekly fill out self-questionnaires regarding the presence of tic exacerbations and experience of common cold. In addition, six throat swabs were taken at fixed intervals irrespective of symptoms, and cultured for streptococci; also, three serial serum assessments of streptococcal antibodies were performed.*

Results: *In the pediatric group, the self-report of a common cold was strongly associated with an exacerbation in tic severity 4 weeks later (odds ratio=4.685, p=0.001). In this group, no association between the self-report of a common cold and tic exacerbations was found in other weeks. In the adult group, we found no association at all between reports of common cold and tic exacerbations. Association with streptococcal infections could not be determined due to the limited number of observed streptococcal infections.*

Conclusion: *While it remains to be proven whether or not streptococcal infections are associated with exacerbations in tic severity, this study points to a hitherto unknown association of common viral infections with exacerbations in tic severity in children, which may support the involvement of immune dysregulation in tic disorders.*

Introduction

Though the precise mechanism at a molecular level is unknown, a growing number of studies suggest the involvement of autoimmunity in the pathogenesis of tic disorders.[1] A common feature of autoimmune disorders in general is their relapsing course over time. Infections have been suggested to induce or reinforce autoimmune reactions in genetically predisposed individuals, and may, thus, be associated with exacerbations and remissions in autoimmune conditions.[2]

A pattern of fluctuations in symptom severity is also common in tic disorders.[3] Some authors have suggested an association between infections with group A beta-hemolytic streptococci and changes in tic severity in at least a subgroup of patients.[4] This possible relationship has been exclusively based on cross-sectional data[5-7] and some case studies,[8-11] however. In the present prospective longitudinal study, we aimed to examine the possible temporal relationship between symptom exacerbations and preceding infections in an unselected cohort of pediatric and adult patients with a tic disorder.

Methods

Subjects

Participants were required to be at least 7 years old. Given the large range in age of participating subjects, we decided to separately analyze patients below and above 16 years old, that is, a pediatric group of patients between 7 and 15 years, and an adult group of 16 years and older. All patients with a tic disorder could only enter the study if they fulfilled the criteria for a definite tic disorder according to the Tourette's Syndrome Research Criteria.[12] These criteria require observable tics to be present during the clinical interview to allow for study entry. Excluded from the study were subjects with a history or family history of an autoimmune disorder. None of the subjects who were willing to participate in the study fulfilled any of these exclusion criteria.

The tic disorder patients were recruited from the outpatient clinic of the Child and Adolescent Psychiatry Center, Groningen, the Netherlands, or from the Dutch Tourette's syndrome patients' association. The aim and procedure of the study were fully explained to the subjects before written consent was requested. If the subjects were under 18, the parents were informed as well, and the written informed consent of the parents and the subject's assent were obtained. The study was approved by the Dutch central medical-ethical committee. To assess tic severity at study entry, we used the motor and vocal scores of the Yale Global Tic Severity Scale.[13] The sum of both scores was used as a measure of total tic severity.

Twenty tic disorder patients entered the pediatric study group (14 boys, six girls), ranging in age from 7 to 15 years (mean=12.3, SD=2.5). The adult group of tic disorder patients consisted of 41 patients (25 men, 16 women) and ranged in age between 16 and 64 years (mean=34.1, SD=15.3). The mean total tic severity score

for the pediatric tic disorder patients at study entry was 18.6 (SD=10.0, range=8-50), with a mean motor score of 12.5 (SD=5.2, range=6-25) and a mean vocal score of 6.1 (SD=6.4, range=0-25). For the adult tic disorder group, the mean total tic severity score at study entry was 23.5 (SD=8.9, range=10-50), with a mean motor score of 14.6 (SD=4.4, range=7-25), and a mean vocal score of 8.9 (SD=6.5, range=0-25). The mean total, motor and vocal tic severity of the adult and pediatric study group did not differ significantly (t-test). At study entry, 13 of the 20 (that is, 65%) pediatric tic disorder patients were taking psychotropic medication, that is, an antipsychotic agent (N=8), clonidine (N=3), or a combination of an antipsychotic agent and clonidine (N=2). In addition, 14 of the 41 (that is, 34%) of the adult patients were on medication, being an antipsychotic agent (N=11) or an antidepressive agent (N=3).

Procedure

During 24 consecutive weeks, each week, on a chosen fixed day of the week, participants were asked to fill out a questionnaire. When participants were children, parents were allowed to assist the children in filling out the questionnaire. On this questionnaire, participants had to state whether or not they had experienced a common cold over the past 7 days, as well as whether or not they had experienced symptoms suggestive of a pharyngitis (i.e., a sore throat). Since common cold is such a universal experience, we did not ask for specific symptoms suggestive of a common cold, but simply asked for the presence or absence of a common cold according to the patient's opinion. In addition, they had to fill out whether the tic severity over the past 7 days had stayed about the same, had decreased or increased slightly, or had increased or decreased much, compared to the preceding period of 7 days. Finally, they were asked to record any change in psychotropic medication regime. All questionnaires were to be mailed to us in stamped envelopes on a weekly base.

During this 24 weeks' study period, every 4 weeks, that is, at study entry (week 0), and subsequently at week four, eight, 12, 16, and 20, throat specimens were taken, which were cultured for streptococci. In addition, at three time points, at week four, 12, and 20, blood was drawn for assessing antistreptolysine O (ASO) titer and anti-deoxyribonuclease B (antiDNAse B) titer. The whole study was performed in the period between September 2001 and May 2002.

Definitions of infections and tic exacerbations

A two titerstep rise in either ASO or antiDNAse B or both was regarded as evidence of an experienced streptococcal infection. To determine the precise time of the actual infection, in case of such a two titerstep rise, we used information from the questionnaires regarding symptoms suggestive of a pharyngitis (i.e. a sore throat). A newly acquired positive throat culture, i.e., a positive throat culture that had not been present in the preceding assessment, was considered a streptococcal colonization, provided we did not detect a subsequent two titerstep rise in the levels of the antistreptococcal antibodies. Positive throat cultures that were present at the first as well as subsequent throat swabs were considered carrier states.

Only those reports of common colds which had not been reported in the preceding week, were considered a common cold in the analyses. When a common cold had already been reported in the preceding week, a subsequent report of a common cold was relabelled as "not a common cold". When no information was available regarding the preceding week (this was the case with regard to all first questionnaires, as well as when participants had failed to return the questionnaire in the preceding week), reports of a common cold on the questionnaires were removed from the analyses, i.e., treated as missing values. Thus, only reports of newly acquired common colds during the observation period were used in the statistical analyses.

When participants filled out that their tic severity over the past 7 days had increased much, compared to the preceding period of 7 days, this was considered a tic exacerbation. All other answers to this topic were classified as "no tic exacerbation."

Statistical analysis

In both patient groups, we intended to determine a possible association of the self-report of a common cold, and a streptococcal pharyngitis or colonization, respectively, with an exacerbation of tic severity during the same week, as well as 1, 2, 3, 4, 5, and 6 weeks later. For this purpose, odds ratios were computed, including 95% confidence intervals. Odds ratios were also computed with regard to a possible association between a change in medication, and tic exacerbations. Two-tailed P values less than 0.05 were considered significant.

Results

Response rate

The pediatric group returned a total number of 412 questionnaires, a response rate of 85.8% returned questionnaires. The adult patient group returned a total number of 731 questionnaires, an overall response rate of 74.3%. In contrast to the pediatric group, in which all patients participated throughout the whole study period, six of 41 adult patients decided to withdraw from the study, all within the first 4 weeks, thus, lowering the overall response rate. Without these six patients, the response rate in the adult group was 84.5%. In addition, we collected a total number of 53 pediatric serial sera for assessment of streptococcal antibodies (88.3% of planned assessments; two of the children refused blood draws), as well as 111 serial adult sera (90.2% of planned sera). Finally, we collected 116 serial pediatric throat swabs (96.7% of planned swabs) as well as 217 serial throat swabs in the adult patient group (88.2% of planned swabs).

Frequency of tic exacerbations

The pediatric patient group reported 55 tic exacerbations, this is 13.3% of all 413 weekly questionnaires, or a mean number of 3.2 exacerbations per child per 24 weeks, whereas the adult patient group reported 99 tic exacerbations, being 13.5% of

731 questionnaires, or a mean number of 3.2 exacerbations per 24 weeks per adult participant.

Frequency of reports of infections

In the pediatric group, 37 common colds were reported, this is 9.6% of valid cases, or a mean number of 2.3 common colds per child per 24 weeks. In addition, in the pediatric group, only one streptococcal infection as well as one single newly acquired streptococcal colonization was detected. Two additional subjects in the pediatric group turned out to be streptococcal carriers.

In the adult group, 58 common colds were reported, this is 8.4% of valid cases, or a mean number of 2.2 common colds per adult participant per 24 weeks. In the adult group, no evidence of a streptococcal infection was detected. A new streptococcal colonization during the study period happened in two adult patients, whereas we found one adult streptococcal carrier state.

Changes in medication

In the pediatric group, participants reported 35 times a change in use of psychotropic medication, or in 8.8% of valid cases. In the adult group, this was reported 75 times, or in 10.6% of valid cases. In both the pediatric and the adult patient group, no statistically significant relationship was found between change in medication use and report of a tic exacerbation during the same week, or 1, 2, 3, 4, 5, or 6 weeks later.

Association between tic exacerbations and infections

In the pediatric group, self-report of a common cold was strongly associated with an exacerbation in tic severity 4 weeks later (table 1). When no common cold was reported, tic severity 4 weeks later was reported "much increased" in 11.2% of cases, whereas the report of a newly acquired common cold led to a "much increased" tic severity 4 weeks later in 37.0% of cases, yielding an odds ratio of 4.685. No statistically significant associations were found between common cold and tic exacerbations in the same week, or 1, 2, 3, 5, or 6 weeks later (table 1). In the adult patient group, no statistically significant associations were observed between reports of a common cold and tic exacerbations in the same week or subsequent weeks (table 2). Given the low number of observed streptococcal infections (N=1 in the pediatric group versus N=0 in the adult group) or colonizations (N=1 in the pediatric group versus N=2 in the adult group), we were not able to determine associations between streptococcal pharyngitis or colonization and subsequent tic exacerbations.

Table 1. *Odds ratios, including 95% confidence intervals and significance levels regarding the association between the self-report of a common cold and tic exacerbations in the same week as well as in the following 6 weeks in the pediatric patient group.*

Week in which exacerbation was reported	***Odds ratio*** *(95% confidence interval)*	***p value***
Same week	0.550 (0.162-1.860)	.301
1 week later	2.219 (0.886-5.554)	.107
2 weeks later	0.876 (0.248-3.092)	.835
3 weeks later	1.684 (0.593-4.783)	.348
4 weeks later	4.685 (1.954-11.23)	.001
5 weeks later	0.402 (0.092-1.765)	.176
6 weeks later	0.768 (0.218-2.708)	.673

Table 2. *Odds ratios, including 95% confidence intervals and significance levels regarding the association between the self-report of a common cold and tic exacerbations in the same week as well as in the following 6 weeks in the adult patient group.*

Week in which exacerbation was reported	***Odds ratio*** *(95% confidence interval)*	***p value***
Same week	1.964 (0.995-3.876)	.065
1 week later	1.267 (0.571-2.808)	.569
2 weeks later	1.406 (0.599-3.304)	.448
3 weeks later	1.989 (0.936-4.229)	.089
4 weeks later	0.934 (0.352-2.477)	.890
5 weeks later	0.983 (0.369-2.623)	.973
6 weeks later	1.491 (0.624-3.563)	.385

Discussion

Although the first notion of a possible link between infection and tic disorders dates back to 1929,[14] this is the first study that examined the association between exacerbations in tic severity and preceding infections in a prospective longitudinal design. In the pediatric patients, we found a strong association between self-reports of a common cold and a subsequent exacerbation in tic severity 4 weeks later. It is improbable that this is due to a nonspecific stress reaction, since we did not find an association with tic exacerbations in other weeks, including the week in which the common cold was newly reported. The usual duration of cold symptoms in children is 10 to 14 days,[15] thus, the cold has already entirely disappeared by the time of the tic exacerbation, 4 weeks later. In contrast, we did not encounter this association in the adult patients. Only a trend regarding the occurrence of tic exacerbations 3 weeks after the cold may be noticeable in adults. Apparently, children and adults differ with regard to the impact of infections on changes in tic severity. While differences between children and adults are well-known with regard to both the

immunological response to infections and the maturity of the nervous system, at present, we do not have a plausible explanation for the different impact of upper respiratory infections on children and adults with a tic disorder. Future studies should focus on the immunological pathways that may be involved. Also, speculations about why the time frame between the upper respiratory infection and the subsequent exacerbation in tic severity appears to be 4 weeks, will have to await such studies. Currently, we know of one additional study[16] that pointed to the relevance of common colds in obsessive-compulsive and tic disorders. In that study, the presence of common cold at the time of onset of obsessive-compulsive disorder and tic symptoms appeared to be associated with sudden, rather than insidious onset of symptoms.

Prior studies pointed to a possible association between tic disorders and streptococcal infections, as was suggested by increased serum levels of antistreptococcal antibodies in unselected patients with a tic disorder.[5-7] Though that approach bears the risk of circular reasoning, other authors preferred to preselect patients based on working criteria for pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections (PANDAS).[17] Indeed, a number of prospectively identified PANDAS cases have recently been presented.[8] Thus, there is some indication that streptococcal infections may be associated with tics. We intended to address this issue in the present study, but identified only one streptococcal infection in the pediatric cohort versus none in the adult patients group over a period of 16 weeks. This frequency of streptococcal infections in our patient groups is in accordance with a reported annual incidence of group A streptococcal throat infections of 36 per 1000 in the general population.[18] In contrast, a previous study[19] prospectively identified 35 streptococcal infections, albeit with a somewhat less stringent definition, during 8 months in 37 children fulfilling PANDAS criteria, despite use of penicillin during 4 of these 8 months. That finding suggests the possibility that children with tics and related disorders would be more susceptible to streptococcal infections. Also, the studies of Muller[5,6] and Cardona[7] would be in accordance with a possibly more frequent incidence of streptococcal infections in tic disorder patients. We were not able to address the issue whether or not streptococcal infections are associated with subsequent exacerbations in tic severity. However, the data of the present study do not appear to support a more frequent occurrence of streptococcal infections in tic disorder patients, compared to the general population. Thus, given the relatively low incidence of streptococcal infections, their role in clinical practice appears to be rather limited.

In contrast, common colds are fairly frequent, with an annual incidence of around four colds per year in teenagers and adults, which center around the winter season in temperate climates.[15] Thus, the present study's reported frequency of slightly more than two colds per individual during the 24 weeks' study period in the fall and winter period, fits well to established epidemiological figures.[15] Common cold is almost exclusively a viral disease.[20] Viral infections are well known to trigger autoimmune conditions,[21-24] though the precise mechanism of induction of autoimmunity by viral infections is largely unknown. Since common colds can be caused by a plethora of viral species, future studies should try to identify which

viruses may be associated with tic exacerbations in children, and how these may influence antineuronal antibody levels.[25] Also, it would be of interest to prospectively study the role of infections regarding the onset of tic disorders, eg in young children with one or both parents and/or siblings affected by tics.

In the present study, we exclusively relied on subjective self-questionnaires for both detecting tic exacerbations as well as common colds. Thus, future studies should try to objectively determine exacerbations in tic severity, using established rating instruments,[3] preferably with at least weekly assessments of tic severity. In addition, such studies may want to objectively document symptoms of common cold. However, the excellent response rates in our study over a period of 24 weeks surely is an indicator of the high motivation of participants, and thus, of the reliability of the present data.

In conclusion, the present finding that common cold appears to be associated with children's exacerbations in tic severity 4 weeks later, adds to the growing literature that indicates the possible involvement of the immune system in tic disorders.[1] In addition, this finding underlines the unique possibilities of tic disorders to study the complex interplay between immune factors, brain functioning and behavior.

References

1. Hoekstra PJ, Kallenberg CG, Korf J, *et al.* Is Tourette's syndrome an autoimmune disease? *Mol Psychiatry* 2002;7:437-45.
2. Luppi P, Rossiello MR, Faas S, *et al.* Genetic background and environment contribute synergistically to the onset of autoimmune diseases. *J Mol Med* 1995;73:381-93.
3. Lin H, Yeh CB, Peterson BS, *et al.* Assessment of symptom exacerbations in a longitudinal study of children with Tourette's syndrome or obsessive-compulsive disorder. *J Am Acad Child Adolesc Psychiatry* 2002;41:1070-7.
4. Swedo SE, Leonard HL, Garvey M, *et al.* Pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections: clinical description of the first 50 cases. *Am J Psychiatry* 1998;155:264-71.
5. Muller N, Kroll B, Schwarz MJ, *et al.* Increased titers of antibodies against streptococcal M12 and M19 proteins in patients with Tourette's syndrome. *Psychiatry Res* 2001;101:187-93.
6. Muller N, Riedel M, Straube A, *et al.* Increased anti-streptococcal antibodies in patients with Tourette's syndrome. *Psychiatry Res* 2000;94:43-9.
7. Cardona F, Orefici G. Group A streptococcal infections and tic disorders in an Italian pediatric population. *J Pediatr* 2001;138:71-5.
8. Murphy ML, Pichichero ME. Prospective identification and treatment of children with pediatric autoimmune neuropsychiatric disorder associated with group A streptococcal infection (PANDAS). *Arch Pediatr Adolesc Med* 2002;156:356-61.
9. Perlmutter SJ, Garvey MA, Castellanos X, *et al.* A case of pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections. *Am J Psychiatry* 1998;155:1592-8.
10. Tucker DM, Leckman JF, Scahill L, *et al.* A putative poststreptococcal case of OCD with chronic tic disorder, not otherwise specified. *J Am Acad Child Adolesc Psychiatry* 1996;35:1684-91.
11. Allen AJ, Leonard HL, Swedo SE. Case study: a new infection-triggered, autoimmune subtype of pediatric OCD and Tourette's syndrome. *J Am Acad Child Adolesc Psychiatry* 1995;34:307-11.
12. Kurlan R. Diagnostic criteria for genetic studies of Tourette syndrome. *Arch Neurol* 1997;54:517-8.
13. Leckman JF, Riddle MA, Hardin MT, *et al.* The Yale Global Tic Severity Scale: initial testing of a clinician-rated scale of tic severity. *J Am Acad Child Adolesc Psychiatry* 1989;28:566-73.
14. Selling L. The role of infection in the etiology of tics. *Arch Neurol Psychiatry* 1929;22:1163-71.
15. Owen Hendley J. The common cold. In: Goldman L, Claude Bennett J, eds. *Cecil Textbook of Medicine*. Philadelphia, W.B.: Saunders Company, 2000:1790-2.
16. Giulino L, Gammon P, Sullivan K, *et al.* Is parental report of upper respiratory infection at the onset of obsessive-compulsive disorder suggestive of pediatric autoimmune neuropsychiatric disorder associated with streptococcal infection? *J Child Adolesc Psychopharmacol* 2002;12:157-64.
17. Garvey MA, Giedd J, Swedo SE. PANDAS: The search for Environmental Triggers of Pediatric Neuropsychiatric Disorders. Lessons from Rheumatic Fever. *J Child Neurol* 1998;13:413-23.
18. Centers for Disease Control and Prevention. Group A Streptococcal (GAS) disease. 2001. Internet Report
19. Garvey MA, Perlmutter SJ, Allen AJ, *et al.* A pilot study of penicillin prophylaxis for neuropsychiatric exacerbations triggered by streptococcal infections. *Biol Psychiatry* 1999;45:1564-71.
20. Makela MJ, Puhakka T, Ruuskanen O, *et al.* Viruses and bacteria in the etiology of the common cold. *J Clin Microbiol* 1998;36:539-42.
21. Hill SL, Rose NR. The transition from viral to autoimmune myocarditis. *Autoimmunity* 2001;34:169-76.
22. Mason A. Viral induction of type 2 diabetes and autoimmune liver disease. *J Nutr* 2001;131:2805S-8S.
23. Cooke SP, Rigby SP, Griffiths DJ, *et al.* Viral studies in rheumatic disease. *Ann Med Interne (Paris)* 1998;149:30-3.

24. Panoutsakopoulou V, Cantor H. On the relationship between viral infection and autoimmunity. *J Autoimmun* 2001;16:341-5.

25. Church AJ, Dale RC, Lees AJ, *et al.* Tourette's syndrome: a cross sectional study to examine the PANDAS hypothesis*. J Neurol Neurosurg Psychiatry.* 2003;74:602-7.

Chapter 9

Association of small life events with self-reports of tic severity in pediatric and adult tic disorder patients: a prospective longitudinal study

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Abstract

Objective: *Clinical experience suggests an association between stressful life events and fluctuations in symptom severity of tic disorder patients. The aim of the present study was to examine this possible relationship in a prospective longitudinal design.*

Methods: *Two groups of tic disorder patients were included in this study, a pediatric group between 7 and 16 years (N=25; 24 of whom completed the study) and an adult group of 18 years and older (N=32; 28 of whom completed the study and reported at least one life event). During a 12 weeks' period, participants were asked to weekly fill out self-questionnaires regarding the occurrence of small life events and self-rating of tic severity.*

Results: *In the adult group as a whole, we found a weak but statistically significant correlation between negative small life events and tic severity during the same week ($r=0.268$, $p<0.001$). However, only a minority of individual pediatric (21%) and adult patients (18%) demonstrated significant relationships between the frequency of small life events and tic severity in the same week or 1 week later, with undesirable small life events positively associated with tic severity in some patients, and negatively associated with tic severity in other patients.*

Conclusion: *Contrary to traditional views, in general, life events do not account for changes in tic severity. Only in a minority of tic disorder patients, fluctuations in symptom severity appear to be associated with possibly stressful small life events.*

Introduction

Symptoms of tic disorders typically show a pattern of waxing and waning severity over the course of time.[1] Clinical experience suggests that this fluctuation in symptom severity may be related to stressful life events, such as interpersonal conflicts, financial problems, and problems associated with work. There is a surprising lack of research with regard to the role of stress in tic disorders. One study, involving children and adolescents with Tourette's disorder, identified events causing anxiety, emotional trauma, and social gatherings to be associated with an increase in tics.[2] That study was based on the subjective experience of patients in a cross-sectional retrospective questionnaire. Previously, in a similar approach, involving a larger series of patients, Shapiro and coworkers[3] had identified that, according to the patients' own impression, tics tend to increase with certain environmental factors, such as anxiety and agitation, and tend to decrease with other factors, most notably nonanxious absorption in a task and the presence of strangers. In addition, a recent study investigated the role of psychosocial stress at two timepoints separated by 4 months in a cohort of children and adolescents with Tourette's disorder and/or obsessive-compulsive disorder.[4] It demonstrated that these patients experienced significantly more psychosocial stress than did age-matched healthy controls. Moreover, that study found that symptom severity correlated with severity of experienced daily life stressors, but not with indices of major life events. No other prospective, longitudinal data are available with regard to a possible association of stressful life events and tic symptom fluctuations.

Evidence for links between psychosocial stress and symptom fluctuations has repeatedly been found for a broad range of disorders, particularly infectious[5,6] and autoimmune conditions.[7] The best predictors of changes in symptom severity have consistently been relatively frequent small irritants of daily life, generally referred to as daily hassles.[8] Examples include arguments with friends, being criticized, having problems with (school)work, and being confronted with unexpected financial burdens.

The Inventory of Small Life Events (ISLE) is one well-known and well-validated diary type scale in which the occurrence of such concrete small life events can be scored.[9] It covers events in major areas of life, that is, family, work, leisure, household, financial state, health/illness, nonfamily relations, crime/criminal activity, education, religion, and transportation. A children's version, the Small Events Inventory-Child reports is also available.[10]

We used these scales in a pediatric and adult cohort of tic disorder patients to investigate the possible relationship between weekly reports of small life events and self-ratings of tic severity in a prospective longitudinal design, over a period of 12 weeks. Our hypothesis was that there would be a correlation between small stressful life events and changes in tic severity over time.

Methods

Subjects

Two groups of tic disorder patients were included in this study, that is, a pediatric group of patients between 7 and 16 years, and an adult group of 18 years and older. All patients with a tic disorder could only enter the study if they fulfilled the criteria for a definite tic disorder according to the Tourette's Syndrome Research Criteria.[11] These criteria require observable tics to be present during the clinical interview to allow for study entry, ensuring the inclusion of patients objectively demonstrating tics. Excluded from the study were subjects with a known IQ below 70. None of the subjects who were willing to participate in the study fulfilled this exclusion criterion. Presence of comorbid diagnoses, which we did not record systematically, was no ground for exclusion.

The tic disorder patients were recruited from the outpatient clinic of the Child and Adolescent Psychiatry Center, Groningen, the Netherlands, or from the Dutch Tourette's syndrome patients' association. Subjects were initially recruited by letters sent to a number of patients with a known clinical diagnosis of a tic disorder at our center, and to members of the patients' association. From our child and adolescent psychiatry center, 35% of approached patients were initially interested to participate. The aim and procedure of the study were fully explained to the subjects before written consent was requested. If the subjects were under 18, the parents were informed as well, and the written informed consent of the parents and the subject's assent were obtained. Patients were informed that we intended to study the relationship between small life events and changes in tic severity over time. However, we did not explicitly reveal the specific study hypothesis. The study was approved by the Dutch central medical-ethical committee. To assess tic severity at study entry, we used the motor and vocal scores of the Yale Global Tic Severity Scale, which records tic severity over the past week.[12] The sum of both scores was used as a measure of total tic severity. We decided not to use the impairment rating of the Yale Global Tic Severity Scale, in order to minimize possible confounding with stress ratings, since the impairment ratings may partially reflect perceived psychosocial stress. Diagnostic assessment and rating of tic severity was performed by one of the authors, either P.J.H. or M.P.S., who are both experienced in the diagnosis of tic disorders.

Twenty-five tic disorder patients entered the pediatric study group, 17 from the patients' association, and eight from our outpatient center. All patients from the patients' association had in the past been referred to a mental health service. The adult group of tic disorder patients consisted of 32 patients, 24 from the patients' association, and eight from our outpatient center. Again, all patients from the patients' association had in the past been referred to a mental health service. Table 1 shows demographic data, tic severity, and medication status at study entry of both patient groups.

Table 1. *Patient characteristics regarding tic severity, age, gender, and use of medication at study entry.*

	Pediatric group		*Adult group*	
	N=25		*N*=32	
Symptom severity	**Mean**	**SD (range)**	**Mean**	**SD (range)**
YGTSS motor tics	12.6	5.7 (4-25)	14.4	3.8 (9-25)
YGTSS vocal tics	5.6	5.8 (0-25)	9.0	6.4 (0-25)
YGTSS total score	18.2	9.1 (8-50)	23.4	8.4 (10-50)
Age	13.0	2.7 (7-16)	36.1	14.6 (18-64)
Gender	***N***	**%**	***N***	**%**
male	19/25	76.0	20/32	62.5
female	6/25	24.0	12/32	37.5
Medication status				
none	11/25	44.0	18/32	56.3
clonidine	3/25	12.0	0/32	0
clonidine+neuroleptic	2/25	8.0	0/32	0
neuroleptic	9/25	36.0	11/32	34.4
AD	0/25	0	3/32	9.4

YGTSS= Yale Global Tic Severity Scale; AD=antidepressive agent

Procedure

During 12 consecutive weeks, each week, on a chosen fixed day of the week, participants were asked to fill out a questionnaire. When participants were children, parents were allowed to assist the children in filling out the questionnaire. However, parents and children were instructed that the child's judgement should form the basis of the scores. On this questionnaire, both pediatric and adult participants were asked to weekly rate the severity of their tics on a 10-point scale in which one represents no tics, two represents minimal tics, and 10 represents the worst-ever tics. In addition, for pediatric patients, the questionnaire contained a Dutch translation of the Small Events Inventory-Child Reports.[10] This scale contains 41 different everyday events that are meaningful indicators of the level of stress in the child's life, as well as indicators of positive transactions between the child and the social world. There are 29 desirable items on the scale, whereas the remaining 12 items are undesirable events. Respondents were asked to indicate whether each event happened once, twice, three times, more than three times, or not at all during the preceding week. The weekly questionnaire of the adult participants contained a Dutch translation of the undesirable items of the ISLE,[9] a total of 46 possible negative events covering all major domains of everyday life. The reason we did not include positive events in the adult ISLE is, that we wanted to keep the list short, in order to increase response rates and reliability, since the adult ISLE contains far more items than the pediatric version. Like the pediatric patients, adult respondents were asked to indicate whether each event happened once, twice, three times, more than three times, or not at all during the preceding week. Finally, both pediatric and adult patients were asked to record any change in psychotropic medication regime. We did not ask for reasons of change in medication. All questionnaires were to be mailed to us in stamped envelopes on a weekly base.

Statistical analysis

For the pediatric group, weekly scores of desirable events and undesirable events, respectively, were computed by adding up all reported frequencies, with a response of "more than three times" counted as 4 times. The same was done with regard to weekly scores of undesirable small life events for the adult patients. For each individual participant, Pearson's correlation coefficients were computed between weekly scores of events and weekly ratings of tic severity. In addition, we computed Pearson's correlation coefficients between weekly scores of events and weekly ratings of tic severity for the pediatric and adult group as a whole. For the latter analyses, we used z-scores of tic severity and small life events scores. These z-scores were based on each individual's ratings across the 12 weeks' study period. Also, we performed the same correlation analyses between tic severity and life events score of the preceding week. To investigate whether there is an association between the initial level of tic severity, as rated by the clinician, and frequency of small life events, we computed Pearson's correlation coefficients between Yale Global Tic Severity Scale measures and first week small life events scores, in both the pediatric and the adult group. Also, in both study groups, we looked for an overall relationship between the patients' average number of negative and, in the case of the pediatric patients, also positive life events, and the average subjective tic severity over the study period, again using Pearson's correlation coefficients. In addition, Pearson's correlation coefficients were computed for both patient groups between the total Yale Global Tic Severity Scale baseline scores, and the subjective tic severity ratings from the same week, to get preliminary data on the subjective ratings' validity. Finally, to rule out medication effects, in both the pediatric and adult patient group, we used the paired-sample t-test to test for possible differences in subjective tic severity in the week in which the medication was changed, and subjective tic severity 1, 2, 3, and 4 weeks later. Patients were only part of the analyses when they participated for more than 2 weeks, and reported at least one small life event during the study period. Two-tailed P values less than 0.05 were considered significant.

Results

Response rate

The pediatric group returned a total number of 241 questionnaires, a response rate of 80.3% returned questionnaires. One pediatric patient decided to withdraw from the study after 1 week. Without this patient, the response rate was 83.3%. The adult patient group returned a total number of 317 questionnaires, an overall response rate of 82.6%. One adult patient decided to withdraw after 1 study week, another adult patient stopped after 2 weeks. Without these patients, the response rate of the adult patient group was 87.2%. Finally, the response rate of the 28 adult patients who were part of the statistical analyses was 86.3% (two adult patients did not report a single negative life event over the entire study period, and were removed from the analyses).

Subjective tic severity ratings

The mean subjective tic severity for the pediatric group was 5.1 (SD=2.4, range=1-10). For the pediatric group, the mean difference between the highest and lowest subjective tic severity rating for each individual over the 12 weeks' period was 4.0 (SD=1.4, range=1-8). The mean subjective tic severity for the adult group was 6.0 (SD=1.9, range=1-10). For the adult group, the mean difference between the highest and lowest subjective tic severity rating for each individual over the 12 weeks' period was 3.5 (SD=1.6, range=0-7). Finally, we identified significant correlations between the total Yale Global Tic Severity Scale baseline scores and the subjective tic severity ratings from the same week, in both the pediatric ($r=0.599$, $p=0.002$) and the adult patients ($r=0.503$, $p=0.006$).

Small life events scores

The mean weekly rating of desirable small life events in the pediatric group was 32.2 (SD=18.2, range=0-81), whereas the mean weekly rating of undesirable small life events in the pediatric group was 2.9 (SD=3.9, range=0-20). In the pediatric group, the mean difference between the highest and lowest weekly desirable small life events frequency rating for each individual over the 12 weeks' period was 31.3 (SD=16.1 range=5-70), versus a mean difference between the highest and lowest weekly undesirable small life events frequency of 7.3 (SD=4.5, range=2-20). The first week desirable life events score demonstrated a significant inverse correlation with the baseline vocal ($r=-0.437$, $p=0.029$), and total tic score ($r=-0.447$, $p=0.025$), but not motor tic score ($r=-0.342$, $p=0.095$). No such significant or nearly significant correlations were found between first week negative life events scores and baseline indices of tic severity.

In the adult group, the mean weekly rating of undesirable small life events was 6.6 (SD=7.8, range=0-78). Two adult patients did not report a single undesirable small life event over the entire study period, two other subjects participated for no more than 2 weeks. One of these last subjects reported a high number of undesirable life events regarding this single week. Without these four subjects, the mean weekly rating of undesirable small life events in the adult group was 6.9 (SD=6.7, range=0-35). For the adult group without the aforementioned four subjects, the mean difference between the highest and lowest weekly undesirable small life events frequency rating for each individual over the 12 weeks' period was 11.9 (SD=7.8, range=2-35). In the adult group, no significant or nearly significant correlations were found between first week negative life events scores and baseline indices of tic severity.

Changes in medication

In the pediatric group, participants reported 19 times a change in their use of psychotropic medication, or in 8.1% of all weekly ratings. In the adult group, this was reported 38 times, or in 12.6% of all weekly ratings. In both the pediatric and the adult patient group, no statistically significant relationship was found between subjective tic severity in the week in which the medication was changed and subjective tic severity 1, 2, 3, and 4 weeks later (paired sample t-tests).

Association between subjective tic severity and small life events

Of 24 pediatric patients who returned more than two questionnaires, 19 (or, 79%) did not show any statistically significant correlation between weekly ratings of tic severity and weekly frequencies of desirable small life events or weekly ratings of undesirable life events during the same week or 1 week earlier. Of the remaining five pediatric patients (21% of the total group), two showed a significant relationship between tic severity and positive events during the same week, albeit in opposite directions ($r=-0.723$, $p=0.008$; and $r=0.781$, $p=0.022$), one patient showed a negative relationship between 1 week-lagged positive events and tic severity ($r=-0.535$, $p=0.022$), and another patient showed a significant positive relationship between 1 week-lagged negative events and tic severity ($r=0.607$, $p=0.048$). Finally, one pediatric patient showed a positive correlation between positive life events and tic severity during the same week ($r=0.720$, $p=0.044$), as well as a negative correlation between tic severity and 1 week-lagged negative life events ($r=-0.811$, $p=0.027$). In the pediatric group as a whole, no statistically significant correlation was found between weekly ratings of tic severity and weekly frequencies of desirable small life events or weekly ratings of undesirable life events. Also, no statistically significant correlation was found between weekly ratings of tic severity and weekly frequencies of desirable small life events or weekly ratings of undesirable life events 1 week earlier. Finally, no overall relationship was identified between the pediatric patients' average subjective tic severity over the study period, and their average number of encountered negative ($r=0.229$, $p=0.282$) and positive small life events ($r=0.099$, $p=0.645$).

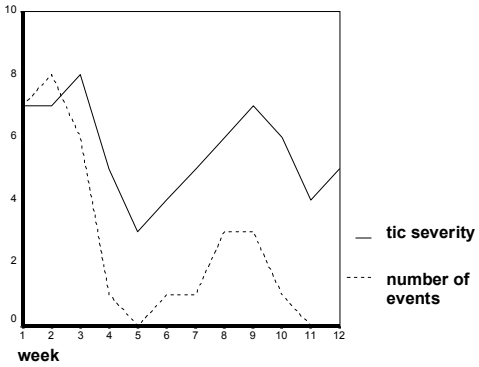
Of the 28 adult patients who participated in the study for longer than 2 weeks and who reported at least one undesirable life event over the 12 weeks' study period, 23 (or, 82%) did not show any statistically significant correlation between weekly ratings of tic severity and weekly frequencies of undesirable small life events during the same week or 1 week earlier. Of the remaining five adult patients (18% of the total group), only one subject showed a significant relationship between tic severity and undesirable life events during the same week ($r=0.818$, $p=0.001$). This same patient also showed a significant positive correlation between tic severity and 1 week-lagged negative life events ($r=0.781$, $p=0.005$). Figure 1 shows the course of tic severity and number of negative small life events of this particular patient. The four remaining patients all showed a significant correlation between tic severity and 1 week-lagged negative small life events, with two patients demonstrating a negative correlation ($r=-.796$, $p=0.006$; and $r=-0.843$, $p=0.002$) and two patients showing a positive correlation ($r=0.964$, $p=0.036$; and $r=0.605$, $p=0.049$). In the adult group as a whole, we found a small, but statistically significant correlation between negative small life events and tic severity during the same week ($r=0.268$, $p<0.001$). In the adult group as a whole, no statistically significant correlation was found between weekly ratings of tic severity and weekly frequencies of undesirable life events 1 week earlier. Finally, no overall relationship was identified between the adults patients' average subjective tic severity over the study period, and their average number of encountered negative small life events ($r=-0.284$, $p=0.143$). Table 2 gives a summary of the main study findings.

Table 2. *Summary of study findings with regard to subjects who participated for more than 2 weeks, and who reported at least one life event during the study period.*

	Pediatric group		*Adult group*	
	N=24		*N=28*	
	N	**%**	***N***	**%**
Returned questionnaires	240	83.3	290	86.3
	Mean	**SD (range)**	**Mean**	**SD (range)**
Subjective tic severity	5.1	2.4 (1-10)	6.0	1.9 (1-10)
Weekly number of positive events	32.2	18.2 (0-81)	n.a.	
Weekly number of negative events	2.9	3.9 (0-20)	6.9	6.7 (0-35)
Relationships with events	***N***	**%**	***N***	**%**
none	19/24	79.2	23/28	82.1
only with negative events	1/24	4.2	5/28	17.9
only with positive events	3/24	12.5	n.a.	
both with negative and positive events	1/24	4.2	n.a.	
Associations within whole group	**r**	***p***	**r**	***p***
negative events		n.s.	.268[1]	.001
positive events		n.s.	n.a.	

n.a.=not available, n.s.=not significant; [1]was found with regard to association between tic severity and number of negative small life events within the same week.

Figure 1. *Course of tic severity and number of experienced negative small life events in an individual adult patient who showed a significant relationship between tic severity and undesirable life events during the same week (r=0.818, p=0.001) and between tic severity and 1 week-lagged negative life events (r=0.781, p=0.005).*



Discussion

Tics have traditionally been considered stress-sensitive phenomena.[13] Indeed, in the adult group, but not in the pediatric study group, we found a weak but statistically significant correlation between negative small life events and changes in tic severity. The results of correlation analyses within individuals, however, indicate that only in a small minority of around 20% of cases, fluctuations in tic severity may be related to stressful small life events. Moreover, in those individuals whose tics appear to be associated with the frequency of reported small life events, the direction of the association may well occasionally be counter-intuitive: we identified individual

patients whose tics deteriorated in association with desirable life events, and other patients in whom undesirable small life events were associated with decrease in tic severity. We also, however, identified individual associations the other way around. Thus, there does not appear to be a general pattern of how stressful life events may be associated with fluctuations in tic severity. This is in accordance with the reports of Silva et al.[2] who also found that tics could decrease in response to a particular environmental factor in some individual patients, which could lead to an increase in tics in other patients. Examples of these ambiguous environmental factors included social gatherings, a doctor's office visit, and reading for pleasure. However, it should be stressed that only in the pediatric group we investigated the role of positive events.

In the present study, we focused on weekly changes in tic severity versus changes in the weekly frequencies of small life events. Indeed, the patients in this study showed rather large weekly fluctuations in self-reported tic severity. Still, in general, the frequency of reported life events, which showed similarly large weekly fluctuations, do not appear to account for the reported changes in tic severity. Despite the apparently limited role of small life events with regard to weekly fluctuations in tic severity, certain psychologically relevant environmental factors, especially anxiety-producing events may still be associated with short term increases in tic severity, which may only last minutes to hours and may not influence tic severity across the better part of a week. Future studies should specifically address that issue using prospective designs.

Other environmental factors may play a more profound role, presumably common infections.[14] It would be of interest to determine whether individual patients can be identified according to a unique responsiveness to environmental factors, such as life events or streptococcal infections.[15] Also, it would be of interest to study biological substrates of stress influences, including cortisol and the role of the hypothalamic-pituitary-adrenal axis.[16]

In future designs, frequency of small life events may be measured simultaneously with perceived stress,[17] since individuals may differ with respect to the impact of small life events. The recent study by the Yale group[4] demonstrated that patients with Tourette's disorder, compared with unaffected controls, greatly differed with regard to reported cross-sectional levels of perceived stress, including perceived severity of stress associated with everyday problems. Patients were not found to differ significantly from controls, however, with regard to the number of experienced life events, as long as discrete events were concerned. While we did not obtain weekly small life events scores from healthy controls, we did not generally encounter significant correlations between the frequency of small life events and baseline tic severity as rated by the clinician. Taken together, the findings of Findley et al.[4] and the present study suggest that tic disorder patients may not differ from unaffected controls with regard to the probability of having encountered life events, but with regard to the levels of experienced stress associated with them. Findley et al.[4] found a significant longitudinal relationship between experienced severity of stress associated with daily life hassles and symptom severity. According to the present study, life events per se are in general not associated with changes in

tic severity. However, it may well be that tic disorder patients are more sensitive to stress associated with daily hassles and that the findings of the study of Findley et al.[4] may in fact reflect this as a consequence, rather than as a cause of changes in tic severity over time. We intended to study the impact of daily life events on tic severity, while trying to rule out the confounding influence of heightened sensitivity to life events, and thus only assessed the frequency of concrete events which all were in principle observable. Unfortunately, direct comparisons of reported small life events scores of the present study, as yielded by the ISLE with previously reported frequencies in other patient groups[18] are hampered by differences in selection of ISLE items and ways of obtaining scores (telephone ratings versus use of self-questionnaires). Thus, future studies should use well-matched control subjects to investigate the issue of frequency of encountered life events and levels of perceived stress associated with them.

Limitations of the study

The relationship between psychosocial stress and tic severity is an important issue, that very few studies have addressed so far. It should be stressed that the present study served primarily exploratory purposes and that it has several limitations, which should be considered in future studies. First, we obtained only a single mean score for baseline tic severity at enrollment. Future studies may consider to use a washout period during which a few weeks of tic fluctuations could be recorded. This might better ground a subsequent response to life events in terms of magnitude. In addition, we failed to investigate the role of major life events,[19] such as breaking up intimate relationships or death of beloved ones. However, the results of the study of Findley et al.[4] seem to indicate a more significant role for psychosocial stress associated with small events than with less frequent major events. Furthermore, in this study, we exclusively relied on subjective self-questionnaires. These correlated modestly with Yale Global Tic Severity Scale scores, what should be viewed, however, against the background that participants subjectively rated their tics according to their individually experienced range of tic severity, rather than according to the overall range of tic severity of tic disorder patients in general. Future studies, though, should try to objectively determine fluctuations in tic severity, using established rating instruments. However, the excellent response rates in our study over a period of 12 weeks surely is an indicator of the high motivation of participants, and thus, of the reliability of the present data.

In conclusion, this study regarding the association between small life events and fluctuations in tic severity confirms the clinical impression that stressful life events may lead to changes in tic severity in individual patients. However, a more important conclusion is, that for the majority of patients this does not appear to be the case. Thus, clinicians should be cautious with simple psychological explanations for exacerbations in symptom severity of tic disorder patients.

References

1. Coffey BJ, Biederman J, Geller DA, et al. The course of Tourette's disorder: a literature review. *Harv Rev Psychiatry* 2000;8:192-198

2. Silva RR, Munoz DM, Barickman JF, et al. Environmental factors and related fluctuation of symptoms in children and adolescents with Tourette's disorder. *J Child Psychol Psychiatry* 1995;36:305-312

3. Shapiro AK, Shapiro ES, Youg GJ, et al. Stimuli-induced changes in severity of symptoms. In: Shapiro AK, Shapiro ES, Youg GJ, et al. Gilles de la Tourette Syndrome, 2nd ed. New York, NY: Raven Press; 1988:179-187.

4. Findley DB, Leckman JF, Katsovich L, et al. Development of the Yale Children's Global Stress Index (YGCSI) and its application in children and adolescents with Tourette's Syndrome and Obsessive-Compulsive Disorder. *J Am Acad Child Adolesc Psychiatry* 2003;42:450-457

5. Leserman J, Petitto JM, Golden RN, et al. Impact of stressful life events, depression, social support, coping, and cortisol on progression to AIDS. *Am J Psychiatry* 2000;157:1221-1228

6. Turner Cobb JM, Steptoe A. Psychosocial influences on upper respiratory infectious illness in children. *J Psychosom Res* 1998;45:319-330

7. Zautra AJ, Hamilton NA, Potter P, et al. Field research on the relationship between stress and disease activity in rheumatoid arthritis. *Ann N Y Acad Sci* 1999;876:397-412

8. Twisk JW, Snel J, Kemper HC, et al. Changes in daily hassles and life events and the relationship with coronary heart disease risk factors: a 2-year longitudinal study in 27-29-year-old males and females. J *Psychosom Res* 1999;46:229-240

9. Zautra AJ, Guarnaccia CA, Dohrenwend BP. Measuring small life events. *Am J Community Psychol* 1986;14:629-655

10. Kanner AD, Feldman SS, Weinberger DA, et al. Uplifts, hassles, and adaptational outcomes in early adolescents. *J Early Adolesc* 1987;7:371-394

11. Kurlan R. Diagnostic criteria for genetic studies of Tourette syndrome. *Arch Neurol* 1997;54:517-518

12. Leckman JF, Riddle MA, Hardin MT, et al. The Yale Global Tic Severity Scale: initial testing of a clinician-rated scale of tic severity. *J Am Acad Child Adolesc Psychiatry* 1989;28:566-573

13. Leckman JF. Tourette's syndrome. *Lancet* 2002;360:1577-1586

14. Hoekstra PJ, Kallenberg CG, Korf J, Is Tourette' syndrome an autoimmune disease? *Mol Psychiatry* 2002;7:437-445

15. Garvey MA, Giedd J, Swedo SE. PANDAS: The search for Environmental Triggers of Pediatric Neuropsychiatric Disorders. Lessons from Rheumatic Fever. *J Child Neurol* 1998;13:413-423

16. Chappell P, Riddle M, Anderson G, et al. Enhanced stress responsivity of Tourette syndrome patients undergoing lumbar puncture. *Biol Psychiatry* 1994;36:35-43

17. Cobb JM, Steptoe A. Psychosocial stress and susceptibility to upper respiratory tract illnessIn nanadult population sample. *Psychosom Med* 1996;58:404-412

18. Zautra AJ, Hoffman J, Potter P, et al. Examination of changes in interpersonal stress as a factor in disease exacerbations among women with rheumatoid arthritis. *Ann Behav Med* 1997;19:279-286

19. Fernandez E, Sheffield J. Relative contributions of life events versus daily hassles to the frequency and intensity of headaches. *Headache* 1996;36: 595-6

Chapter 10

Lack of effect of intravenous immuno-globulins on tics: a double-blind placebo-controlled study

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Abstract

Objective: *Case studies and a placebo-controlled study previously suggested the effectiveness of immunomodulatory therapy in those patients with tic or related disorders whose symptoms show a relationship with streptococcal infections. No data are available on the effectiveness of intravenous immunoglobulins (IVIG) on tic severity in unselected tic disorder patients.*

Methods: *Thirty tic disorder patients were randomized to IVIG (1 g/kg on two consecutive days; mean age of subjects=28.71, range=14-53) or placebo (mean age of subjects=30.73, range=14-63). Symptom rating occurred at baseline and at week two, four, six, 10, and 14 post-treatment, after which blinding was broken.*

Results: *We observed no significant differences between both treatment groups regarding post-treatment changes in tic severity. Severity of obsessions and compulsions, which was in the subclinical range at base line, decreased significantly in the IVIG group compared to the placebo group at week six (p=0.02). Then, there was a 32.3% improvement in the IVIG group compared to base line level. Though this improvement was maintained over the following 8 weeks, no statistically significant differences between the IVIG and the placebo group with regard to improvements in obsessions and compulsions were detected at subsequent assessments. IVIG treatment was associated with significantly more side-effects than placebo, most notably headache.*

Conclusion: *Based on the present results, IVIG cannot be recommended in tic disorders.*

Introduction

Tic disorders, formerly thought as having a psychogenic cause,[1] are now generally regarded neurobiological disorders with a strong genetic component.[2] These disorders are characterized by the presence of recurrent sudden movements and/or utterances, frequently accompanied by associated behavioral difficulties, such as hyperactivity/impulsivity, attentional problems, emotional lability, rage attacks, and obsessive-compulsive symptoms.[3] Though symptoms may improve after the onset of puberty,[4] a considerable number of patients suffer lifelong from this debilitating symptomatology. Intriguing recent research findings support the possible involvement of autoimmunity in tic disorders.[5] Infections are thought to induce symptom exacerbations,[6] possibly through the involvement of antineuronal autoantibodies.[7] The relationship between infections and symptom fluctuations is most evident in cases fulfilling criteria for pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections (PANDAS),[8] but may also apply to a broader range of tic disorder patients, as recently discussed.[5] The relevance of immunological factors may have important treatment implications. Indeed, some published case studies reported the successful application of immunomodulatory therapies in patients with tic disorders, mostly reported in cases fulfilling PANDAS criteria. These included immunosuppression with corticosteroids,[9-11] therapeutic plasma exchange,[12,13] and intravenously administered immunoglobulins (IVIG).[13-15]

Only a single placebo-controlled study is available with regard to immune-based treatments.[16] That study pointed to the effectiveness of both plasma exchange and IVIG in pediatric tic and obsessive-compulsive disorder patients who all met PANDAS criteria,[8] compared to a placebo condition. Interestingly, a single course of plasma exchange or IVIG resulted in positive effects that were still present at 1 year after treatment. Treatment with IVIG resulted in significant improvements in obsessive-compulsive symptoms, anxiety, and depression. However, the IVIG group did not show significant improvement in tic severity. Since the study of Perlmutter et al.[16] included two different disorders, that is, obsessive-compulsive disorder (OCD) and/or tic disorders, the number of patients with tics in that study was rather low (eight children with tics in the placebo group versus four in the IVIG group). In addition, given the PANDAS criteria that subjects had to meet, which require evidence of an association between streptococcal infection and onset or exacerbation of signs and symptoms, the patients who were enrolled in the study may well not be representative of tic disorder patients in general. In other words, whether IVIG benefits tic severity in unselected patients with a tic disorder has not been addressed. This is an important issue, given the possible involvement of immune factors across tic disorder patients who were not specifically preselected using PANDAS.[6,17] Meanwhile, the imposing results of the single placebo-controlled study,[16] as well as the published highly successful case reports,[13-15] information which is readily accessible to a lay public via the internet, has occasionally led to considerable pressure inflicted on clinicians by tic disorder patients or their parents to apply one of the immunomodulatory treatment options.

Also, many clinicians themselves wonder if they should refer their patients with tic or related disorders to such treatment modalities, especially given the paucity of currently available treatment possibilities, which consist largely of the use of antipsychotic agents, that are purely symptomatic and may have troublesome side-effects. This led us to conduct the present double-blind study in which we compared the therapeutic effect of a single course of IVIG with a placebo condition in a group of unselected patients with a chronic tic disorder. We used changes in tic severity as the primary endpoint of the study. In addition, we assessed changes in obsessive-compulsive symptoms.

Contrary to the study of Perlmutter et al.,[16] who studied children aged 5-14 years, we decided to enroll patients of 14 years and older, including adult patients. Spontaneous, long lasting remissions in tic severity are far less likely to occur in older patients, especially adults, than in prepubertal children.[18] Thus, development of effective treatment options is most urgently needed for those older patients, who continue to show bothersome tics. We hypothesized that IVIG treatment would be more effective than placebo in lessening severity of tics and obsessive-compulsive symptoms.

Methods

Patient recruitment

Patients with a tic disorder of 14 years or older were recruited from two sources, that is, from members of the Tourette's syndrome patients' association in the Netherlands and from patients who had been referred to the outpatient clinic of the Child and Adolescent Psychiatry Center in Groningen, the Netherlands. Patients of both groups received a written invitation to participate in the study. This letter provided information about the aims and background of the study. A total number of 64 patients (21 from our outpatient clinic and 43 from the patients' association) were initially interested to participate and were assessed for study eligibility by the first author. Eligibility criteria included fulfillment of the research criteria for a definite tic disorder according to the Tourette's Syndrome Classification Study Group[19] (implying that observable tics have to be present during the clinical interview to allow for study entry) and presence of tics severe enough to cause significant distress and interference with the patient's functioning in at least two spheres (home, school, work, social relationships). This tic severity requirement was based on the subjective experience of patients and not on a formal tic rating cutoff. In addition, the presence of a tic disorder had to be the primary problem. Though none of the patients met PANDAS criteria,[8] these were not used as an exclusion criterion. Excluded from the study were subjects with total IgA deficiency and anti-IgA antibodies, given the risk of anaphylactic reactions caused by IgE class anti-IgA antibodies reacting with IgA in the IVIG preparation. Therefore, all patients were screened with regard to IgA deficiency. This was detected in none of the subjects who were willing to participate. The aim and procedure of the study were fully explained to the subjects before written consent was requested. If the

subjects were under 18, the parents were informed as well, and the written informed consent of the parents and the subject's assent were obtained. The study was approved by the Dutch central medical-ethical committee (The Hague, the Netherlands) and was in accordance with the Helsinki Declaration of 1975, as revised in 1983. Thirty-four patients did not meet inclusion criteria or were unwilling to give final consent; the remaining 30 patients (eight from our outpatient clinic and 22 from the patients' association) were subjected to randomization. All patients from the patients' association had in the past been referred to a mental health service. Thus, all participating patients can be regarded as referred patients.

Treatment

The 30 tic disorder patients were en bloc randomized to IVIG or placebo with stratification by sex and age (above and below 18 years). Treatment of all patients was subsequently scheduled within a period of 14 weeks onward. Patients allocated IVIG treatment received 1 gram per kilogram immmunoglobulins (Gammagard, Baxter, the Netherlands) daily for two consecutive days. Gammagard is a sterile, freeze-dried preparation of at least 90% IgG, purified from large pools of human plasma, stemming from at least 1 000 donors. The placebo infusion consisted of an equal volume of 5% albumin solution in the same vehicle prepared by the manufacturer of the immunoglobulins, which was also administered daily for two consecutive days. Investigators, nurses, and patients were unaware of the treatment assignments.

All treatments took place at the day care facility of the University Hospital Groningen, the Netherlands. After an initial dose of 0.5 mg per kilogram per minute over a period of 30 minutes, the infusions were administered at a rate of 3 mg per kilogram per minute over a period of 5 to 6 hours. Vital signs, including blood pressure, were monitored throughout the infusion.

The first investigator, who performed all pre- and post-treatment psychiatric assessments, was kept unaware of the occurrence of side-effects, which might have revealed the active treatment. Patients were explicitly asked, not to discuss these with the first investigator at all assessments. Mild side-effects were to be treated symptomatically. Acetaminophen was to be used in the case of headache, flu-like symptoms, and fever, whereas mild adverse reactions (simple urticaria) were to be treated symptomatically with antihistamines. Treatment-resistant headache, flu-like symptoms, or fever would lead to a maximum 24-hour interruption of study medication. Repeated adverse events would cause discontinuation of treatment. Side-effects were recorded by each patient on a form containing possible IVIG-associated side-effects.

Throughout the trial, patients were free to continue or adjust any neuropsychiatric medication, as appropriate according to their physicians, with no limits on the permissible dose adjustment. This was done for ethical reasons, given the unproven effectiveness of IVIG.

Evaluation

Changes in tic severity as assessed by the Yale Global Tic Severity Scale[20] were the primary endpoint of the study. This scale provides an evaluation of the number, frequency, intensity, complexity, and interference of motor and phonic symptoms, based on direct examination and historical data from multiple informants. In addition, we assessed post-treatment changes in severity of obsessions and compulsions.

Standardized ratings of neuropsychiatric signs and symptoms were obtained for each patient at baseline. These consisted of the Yale Global Tic Severity Scale (of which we only used the sum of the motor and vocal tic score), the Yale-Brown Obsessive Compulsive Scale,[21,22] or the children's version[23] for subjects below 16 years, and the clinical global impression scale of symptom change with regard to tic severity.[24] For the statistical analyses with the latter scale, we decided to combine the categories "very much improved" and "much improved" to the outcome "treatment response" and to label all other categories, that is, "minimally improved", "no change", "minimally worse", "much worse", and "very much worse" to one category, "no treatment response". Evaluations of severity of neuropsychiatric signs and symptoms were scheduled at week two, four, six, 10, and 14 after treatment. Serum creatinine was measured at baseline and on day three. After the final evaluation of the last patient, the IVIG/placebo masking was broken en bloc. Results were analyzed on the basis of intention to treat. Patients were not asked which treatment they thought they had received.

Statistical analysis

To measure differences between groups at baseline and subsequent assessments after treatment, we used repeated-measures analysis of variance (ANOVA) on each of the neuropsychiatric ratings by use of the SPSS statistical program. Differences in outcome of the clinical global impression scale of symptom change between both treatment groups were tested by chi-square analysis. Chi-square analysis was also used to test for differences in the occurrence of side-effects. Differences in baseline severity between both treatment groups were assessed by the Mann-Whitney U test. All tests of significance used the 0.05 level of significance and were two-tailed.

Results

Patient characteristics

One scheduled patient chose to withdraw from the trial before treatment. This subject had been randomized to receive IVIG. Thus, 14 patients received IVIG and 15 patients placebo. Patient characteristics of the two randomized groups are shown in table 1. Both groups were comparable in age, sex, tic disorder diagnosis, and use of psychotropic medication. At baseline, tic severity and severity of obsessions and compulsions did not differ significantly. None of the patients fully met DSM-IV criteria for OCD; severity of obsessions and compulsions was in the subclinical range. No patients were lost on follow-up.

Table 1. *Patient characteristics at baseline.*

	IVIG *N*=14		*Placebo* *N*=15	
	Mean	**range**	**Mean**	**range**
Age	28.71	14-53	30.73	14-63
Gender	***N***	**%**	***N***	**%**
male	9/14	64.3	9/15	60.0
female	5/14	35.7	6/15	40.0
Medication status				
none	6/14	42.9	7/15	46.7
neuroleptic	3/14	21.4	7/15	46.7
neuroleptic+AO	2/14	14.3	0/15	0.0
AO	3/14	21.4	1/15	6.7
Symptom severity	**Mean**	**SD**	**Mean**	**SD**
YGTSS	25.0	9.6	25.5	8.9
YBOCS	10.2	9.2	5.6	7.8
Type of tic disorder	***N***	**%**	***N***	**%**
TD	13/14	92.9	13/15	86.7
CMT	1/14	7.1	2/15	13.3

IVIG=intravenous immunoglobulins; AO=anti-obsessional agent; YGTSS= sum of motor and vocal score on Yale Global Tic Severity Scale; YBOCS=score on Yale-Brown Obsessive Compulsive Scale, or children's version for subjects below 16 years; TD=Tourette's disorder; CMT=chronic motor tic disorder;

Treatment response

No significant differences were observed between both treatment groups with regard to post-treatment changes in tic severity (table 2). In both the IVIG group and the placebo group, post-treatment ratings of tic severity were slightly lower compared to baseline, that is, between 2.4% and 19.6% for the IVIG group and 4.7% and 16.5% for the placebo group, depending on the post-treatment week. Regarding changes in severity of obsessions and compulsions, a significant difference between both treatment groups was only observed at week six (repeated measure ANOVA; $F=5.7$; $p=0.02$). At that time point, there was a 32.3% improvement in the IVIG group compared to the base line level. In contrast, in the placebo group the improvement at week six was 3.6% compared to baseline. Though improvement in obsessions and compulsions was maintained over the following 8 weeks (table 2), no statistically significant differences between the IVIG and the placebo group with regard to improvements in obsessions and compulsions were detected at subsequent assessments.

Treatment response, as determined by the clinical global impression scale of symptom change ranged between 6.7% and 33.3% in both treatment groups at the different post-treatment time points. Chi-square analysis did not reveal differences in number of responders between treatment groups (table 2). Four patients recorded changes in the use of psychotropic medication during the trial. Two patients stopped their haloperidol medication, which they both had used in a dose of once daily 1 mg. One other patient started to use haloperidol, also in a dose of 1 mg daily. The final patient who recorded medication changes had lowered his

risperidone medication by 1 mg, while at the same time increasing his haloperidol dose by 1 mg. All patients who changed their medication fell in the placebo group. The changes in psychotropic medication did not lead to the category "treatment response" in all four cases, however.

Table 2. *Mean symptom severity at baseline and at different post-treat-ment points, compared to baseline, as well as percentages of treatment responders.**

Rating	*Baseline*		*Week 2*			*Week 4*		
	IG	**Pl**	**IG**	**Pl**	**p**	**IG**	**Pl**	**p**
YGTSS	25.0	25.5	22.9	22.5	.77	24.4	21.3	.17
YBOCS	10.2	5.6	6.1	4.5	.11	9.2	3.7	.50
% responders			7.1	33.3	.08	7.1	33.3	.08

Rating	*Week 6*			*Week 10*			*Week 14*		
	IG	**Pl**	**p**	**IG**	**Pl**	**p**	**IG**	**Pl**	**p**
YGTSS	21.1	22.4	.77	22.1	24.3	.40	20.1	24.3	.18
YBOCS	6.9	5.4	.02	6.4	4.6	.13	6.7	4.5	.11
% responders	14.3	26.7	.41	21.4	13.3	.56	28.6	6.7	.12

IG=IVIG (intravenous immunoglobulins); Pl=placebo; YGTSS=sum of motor and vocal score of Yale Global Tic Severity Scale (range=0-50); YBOCS=score on Yale-Brown Obsessive Compulsive Scale, or children's version for subjects below 16 years (range=0-40)

*Treatment response was based on the presence or absence of a "very much" or "much improvement" rating according to the clinical global impression scale of symptom change, with regard to tic severity. P values represent significance levels of repeated-measures analyses of variance with regard to differences in treatment response between patients who received IVIG treatment and patients who received placebo, and significance levels of chi-square analyses regarding differences in percentages of treatment responders between treatment groups, respectively.

Side-effects of treatment

Mild-to-moderate side-effects were reported by four patients receiving placebo (27%) versus 13 of 14 patients receiving IVIG (93%). Table 3 shows the most frequently observed side-effects. Most side-effects tended to occur during the second day of the infusion. The use of acetaminophen was greater in the IVIG-group than in the placebo-group (71% versus 27%; Pearson's chi-square=5.8, p=0.016). Antihistamines for treating adverse reactions were used in three patients (21%), all belonging to the IVIG-group. Severe treatment-resistant headache led to a 24-hour interruption of medication on the second day in one patient, which implied that the rest of the dose was given on a third day. This patient belonged to the IVIG-group. Due to treatment-resistant difficulty in breathing, one patient, who belonged to the IVIG-group, discontinued treatment on the second day of the infusion. This patient had received 1 g/kg IVIG on the first day, as scheduled, and had finished 12.5% of the planned amount of IVIG on the second day. The first investigator, who performed all pre- and post-treatment psychiatric assessments, never became aware of the occurrence of adverse events before deblinding.

Table 3. *Occurrence of mild-to-moderate side-effects in both treatment groups. P values represent significance levels for differences between groups (Pearson's chi-square).*

	IVIG *N*=14		*Placebo* *N*=15		
	N	**%**	***N***	%	**P**
Any side-effects	13	93	4	27	<.001
Chills	6	43	1	7	.023
Headache	11	79	4	27	.005
Fever	5	36	0	0	.011
Vomiting	4	29	0	0	.026
Nausea	7	50	1	7	.009
Dizziness	3	21	0	0	.058

Discussion

This is the first double-blind placebo-controlled study in which the effect of IVIG is examined in unselected chronic tic disorder patients, either Tourette's disorder or chronic motor tic disorder, two etiologically closely related disorders.[25] According to our data, IVIG does not appear to be an effective treatment with regard to reducing tic severity in these patients. One major difference with the earlier study of Perlmutter et al.[16] is our use of a patient group, whose main feature was the presence of tics, while the study of Perlmutter et al.[16] involved children who did or did not have tics, and whose primary problems were in the field of obsessions and compulsions and emotional problems, including anxiety and depressive symptoms. A second major difference is that our patients did not meet PANDAS criteria contrary to the study of Perlmutter et al.[16] Finally, the present study involved adolescent and adult patients, as opposed to the pediatric patients in the study of Perlmutter and colleagues.[16] Still, regarding effect on tic severity, our results are in accordance with the latter study,[16] which, also, did not show improvement in tic severity after IVIG.

Apart from the possibility that IVIG may have no effect on tics, a number of other factors may explain the lack of treatment response in the present study. First, patients in our study had experienced many years of disease, which may be associated with non-reversible damage to relevant neuronal circuits. Furthermore, patients were not preselected with regard to a presumed autoimmune etiology, based on either clinical criteria[8] or laboratory parameters.[26] The present study cannot rule out that highly selected patients may still profit from treatment with IVIG. Moreover, the patients had access to psychotropic medication both prior to and during the trial, and thus form a treated patient population. This makes it more difficult to detect a positive effect from a new treatment, which increases the risk of a type II error. Another risk for a type II error may be the relatively low number of patients in each treatment arm. Finally, we used only a

single IVIG dose, as did Perlmutter and coworkers,[16] whereas many IVIG treatment protocols use repeated, often monthly IVIG applications over time.[27] It may still be that multiple IVIG doses doses over a longer period of time appear to demonstrate efficacy in lessening of tic severity. However, the present available data from both our and Perlmutter's study[16] do not lend support to the application of IVIG in tic disorder patients.

Though we studied patients with a primary diagnosis of a tic disorder, we did find a significant effect regarding improvement of obsessive-compulsive symptoms in the IVIG group. At week six post-treatment, ratings of obsessions and compulsions had been decreased by 32% in the IVIG group. While this improvement was maintained over the following 8 weeks, differences between the IVIG and the placebo group with regard to improvements in obsessions and compulsions did not reach statistical significance at subsequent assessments.

Also in the study of Perlmutter et al.,[16] IVIG appeared to benefit severity of obsessions and compulsions. Thus, there is some indication that IVIG may improve obsessions and compulsions. Still, the present results regarding possible effectiveness of IVIG for symptoms of OCD should be viewed with much caution, given the fact that obsessions and compulsions were by no means the patients' main symptoms. In fact, baseline ratings for OCD symptoms were rather low in our study and were in the subclinical range. Thus, observed improvements should not be considered clinically significant. In addition, the between-group difference in improvement of OCD symptoms at week six post-treatment may have been primarily related to a floor effect, due to the very low YBOCS baseline ratings in the placebo group. Future studies should specifically study the effect of IVIG in patients with different subtypes of OCD, eg pediatric onset versus adult onset OCD, OCD with tics versus OCD without tics, and OCD with and without poststreptococcal exacerbations.

Contrary to Perlmutter et al.,[16] we found a relatively high placebo response in our study, with 33% of patients in the placebo group being much or very much improved at 2 weeks post-treatment. While we do not have an explanation for the striking lack of placebo effect in the study of Perlmutter et al.,[16] the sizeable placebo response that we encountered, may well explain some of the successes of the case studies which reported improvement after immune-based therapy,[13-15] as well as some of the effect of the plasma exchange in the study of Perlmutter et al.,[16] which was not placebo-controlled. Thus, future studies should use blinded and well-controlled designs.

In conclusion, based on the present results, we cannot recommend IVIG treatment for reducing tic severity. Moreover, at present, the use of IVIG in OCD patients should be confined to placebo-controlled research protocols.

References

1. Kushner HI. A cursing brain? : the histories of Tourette syndrome. Cambridge (MA): Harvard University Press; 1999.
2. Jankovic J. Tourette's syndrome. *N Engl J Med* 2001;345:1184-1192
3. Kurlan R, Como PG, Miller B, et al. The behavioral spectrum of tic disorders: a community-based study. *Neurology* 2002;59:414-420
4. Coffey BJ, Biederman J, Geller DA, et al. The course of Tourette's disorder: a literature review. *Harv Rev Psychiatry* 2000;8:192-198
5. Hoekstra PJ, Kallenberg CG, Korf J, et al. Is Tourette's syndrome an autoimmune disease? *Mol Psychiatry* 2002; 7:437-445
6. Muller N, Kroll B, Schwarz MJ, et al. Increased titers of antibodies against streptococcal M12 and M19 proteins in patients with Tourette's syndrome. *Psychiatry Res* 2001;101:187-193
7. Wendlandt JT, Grus FH, Hansen BH, et al. Striatal antibodies in children with Tourette's syndrome: multivariate discriminant analysis of IgG repertoires. *J Neuroimmunol* 2001;119:106-113
8. Swedo SE, Leonard HL, Garvey M, et al. Pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections: clinical description of the first 50 cases. *Am J Psychiatry* 1998;155:264-271
9. Kondo K, Kabasawa T. Improvement in Gilles de la Tourette syndrome after corticosteroid therapy. *Ann Neurol* 1978;4:387
10. Matarazzo EB. Tourette's syndrome treated with ACTH and prednisone: report of two cases. *J Child Adolesc Psychopharmacol* 1992;2:215-226
11. Geschwind N, Kondo K. Corticosteroid therapy and Tourette syndrome. [letter]. *Ann Neurol* 1979;5:495
12. Tucker DM, Leckman JF, Scahill L, et al. A putative poststreptococcal case of OCD with chronic tic disorder, not otherwise specified. *J Am Acad Child Adolesc Psychiatry* 1996;35:1684-1691
13. Allen AJ, Leonard HL, Swedo SE. Case study: a new infection-triggered, autoimmune subtype of pediatric OCD and Tourette's syndrome. *J Am Acad Child Adolesc Psychiatry* 1995;34:307-311
14. Muller N, Riedel M, Erfurth A, et al. Immunoglobulin therapy in Gilles de la Tourette syndrome. *Nervenarzt* 1997;68:914-916
15. Perlmutter SJ, Garvey MA, Castellanos X, et al. A case of pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections. *Am J Psychiatry* 1998;155:1592-1598
16. Perlmutter SJ, Garvey MA, Castellanos X, et al. Therapeutic plasma exchange and intravenous immunoglobulin for obsessive-compulsive disorder and tic disorders in childhood. *Lancet* 1999;354:1153-1158
17. Murphy TK, Goodman WK, Fudge MW, et al. B lymphocyte antigen D8/17: a peripheral marker for childhood-onset obsessive-compulsive disorder and Tourette's syndrome? *Am J Psychiatry* 1997;154:402-407
18. Bruun RD, Budman CL. The course and prognosis of Tourette syndrome. *Neurol Clin* 1997;15:291-298
19. The Tourette Syndrome Classification Study Group. Definitions and classification of tic disorders. *Arch Neurol* 1993;50:1013-1016
20. Leckman JF, Riddle MA, Hardin MT, et al. The Yale Global Tic Severity Scale: initial testing of a clinician-rated scale of tic severity. *J Am Acad Child Adolesc Psychiatry* 1989;28:566-573
21. Goodman WK, Price LH, Rasmussen SA, et al. The Yale-Brown Obsessive Compulsive Scale. I. Development, use, and reliability. *Arch Gen Psychiatry* 1989;46:1006-1011
22. Goodman WK, Price LH, Rasmussen SA, et al. The Yale-Brown Obsessive Compulsive Scale. II. Validity. *Arch Gen Psychiatry* 1989;46:1012-1016
23. Scahill L, Riddle MA, McSwiggin-Hardin M, et al. Children's Yale-Brown Obsessive Compulsive Scale: reliability and validity. *J Am Acad Child Adolesc Psychiatry* 1997;36:844-852
24. Guy W. In: ECDEU assessment manual for psychopharmacology: US department

of *Health, Education and Welfare*; 1976:217-222.

25. Santangelo SL, Pauls DL, Lavori PW, et al. Assessing risk for the Tourette spectrum of disorders among first-degree relatives of probands with Tourette syndrome. *Am J Med Genet* 1996;67:107-116.

26. Church AJ, Dale RC, Lees AJ, et al. Tourette's syndrome: a cross sectional study to examine the PANDAS hypothesis. *J Neurol Neurosurg Psychiatry*. 2003;74:602-607

27. Levy Y, Sherer Y, George J, et al. Serologic and clinical response to treatment of systemic vasculitis and associated autoimmune disease with intravenous immunoglobulin. *Int Arch Allergy Immunol.* 1999;119:231-238

Chapter 11

Neurobiology of Tourette's syndrome: do immune factors really matter?

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Abstract

Tourette's syndrome is a childhood-onset neuropsychiatric disorder characterized by the presence of both multiple motor and vocal tics. While the pathogenesis at a molecular and cellular level remains unknown, structural and functional neuroimaging studies point to the involvement of the basal ganglia and related cortico-striato-thalamo-cortical circuits as the neuroanatomical site for Tourette's syndrome. Moreover, Tourette's syndrome has a strong genetic component and considerable progress has been made in understanding the mode of transmissioin and in identifying potential genomic loci. Summaries of recent findings in these areas will be reviewed, followed by a critical overview of findings both supporting and challenging the proposed autoimmune hypothesis of Tourette's syndrome. We conclude that Tourette's syndrome is a heterogeneous disorder, and that immune factors may indeed be involved in some patients.

Introduction

A tic is an involuntary, sudden, rapid, recurrent, nonrhythmic, stereotyped motor movement or vocalization.[1] Thus, a distinction is made between motor and vocal tics. Tics are rather common in school age children, but are more often than not transient phenomena.[2] They may, however, follow a chronic course, and can be present lifelong in some persons.[3] The best studied tic disorder is Tourette's syndrome (TS), defined in the latest edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV TR)[1] by the presence of both multiple motor tics and one or more vocal tics throughout a period of more than 1 year, during which period there was never a tic-free period of more than three consecutive months. Closely related,[4] are a number of other tic disorders as defined in DSM-IV TR, most notably chronic motor tic disorder and chronic vocal tic disorder, in which only one type of tic is present, either solely motor movements or vocalizations, respectively.

While tic disorders were once regarded as psychogenic conditions,[5] twin and family studies now indisputably point to a major genetic contribution in these disorders. Neurobiological research has revealed a number of possible alterations and there is some indication that immune dysregulation may be involved in the pathogenesis of tic disorders. Briefly, some of the immune research suggests that infections may induce or reinforce tics and associated features in susceptible individuals, through the possible involvement of abnormal humoral immune responses directed against self-tissue antigens. After outlining the main clinical features of TS and summarizing the research in the areas of genetics, neurochemistry and neuroimaging, we will present an overview of findings supporting, as well as challenging the immune hypothesis of tic disorders.

Clinical features

Tics vary greatly between and within individuals. Most motor tics are brief, sudden and meaningless muscle movements, such as eye blinking, nose twitching, or shoulder shrugging, referred to as simple motor tics. In contrast, complex motor tics appear more purposeful and involve several muscle groups. Examples include touching other people or objects, retracing steps when walking, and various complex hand gestures. Similarly, vocal tics may be subdivided into simple and complex tics, ranging from meaningless sounds as throat clearing, sniffing, and barking to the sudden utterance of words, phrases, and full sentences which may include echolalia and coprolalia.

Tic intensity can vary substantially, ranging from barely visible or audible tics to extremely forceful or loud expressions. Quite often, tics are mild, in which case they may hardly attract attention from others and may not interfere with everyday life at all. However, powerful and frequent tics may severely interfere with everyday activities, including speech, driving, and walking. In exceptional cases, tics may lead to physical injury, including joint dislocation and other tissue

damage. Patients who display more severe or complex tics may be stigmatized as a result of the unusual, inappropriate, and bizarre character of their tics.

Most patients do not experience their tics as entirely beyond their control.[6] Many individuals describe premonitory urges preceding their tics,[7] feelings that are momentarily relieved by the performance of tics and may be temporarily ignored by suppressing the tics. Furthermore, tics tend to occur in bouts alternated with relatively tic-free periods within the course of a day.[8]

The median age of onset of tics is 7 years.[9] Onset before the age of two and after the age of 12 is highly unusual. In general, tics do not start abruptly in an individual's life. More typical is a gradual initial course, in which weeks with mild tics alternate with tic-free periods. Over time, tics may either disappear spontaneously, or may gradually become more frequent and prominent. Initial tics almost invariably involve the face; most frequently, eye blinking forms the first tic symptom. In the following months to years, tics may spread to other body parts and may become more complex and forceful. Vocal tics tend to begin several years after the first motor tics have appeared. They commonly start as meaningless simple sounds, but may be supplemented by more complex vocalizations later on. Especially in children, tics tend to vary substantially over time in their location, frequency, and forcefulness and may typically follow a waxing and waning course with regard to overall severity.[10] This changing pattern tends to become less varied by the early adult years, concomitant with an overall trend of diminishing tic severity[11] with regard to both intensity and frequency. Still, a significant minority of patients may continue to demonstrate bothersome tics in their adult years.

A recent large epidemiological study reported transient tics to be present in up to 5% of school age children and reported a prevalence of TS in school age children of about 0.6%, with males found to be more frequently affected than females.[2] This prevalence of 0.6% is higher than those reported in previous studies.[12]

A final important feature of tic disorders is their association with a wide range of comorbid behavioral abnormalities, which in certain individuals may be more clinically relevant than the tics themselves. Most studies have reported increased frequencies of hyperactivity, impulsivity, attentional impairments, obsessive-compulsive features, and difficulties regarding social functioning in tic disorder patients, when examined on a group basis.[13-15]

Neuroanatomy

Currently, the pathogenesis of tic disorders at a molecular and cellular level is unknown. Also, there is no definite evidence of the neuroanatomical substrate of tics. The basal ganglia play an established role in other movement disorders including Huntington's disease,[16] Parkinson's disease,[17] and encephalitis lethargica,[18] and thus form an attractive candidate site for involvement in tic disorders. Moreover, based on the phenomenology and natural history of tic

disorders, cortico-striato-thalamo-cortical (CSTC) circuits have been suggested to be involved in tic disorders.[19] At least five functionally and anatomically distinct CSTC circuits have been identified, which subserve sensorimotor, motor, oculomotor, cognitive, and limbic processes (recently reviewed by Mink[20]). A failure to inhibit specific CSTC subsets has been hypothesized to be involved in tic and related disorders and may be directly linked to specific types of tics, given the somatotopical organization of these CSTC circuits. For example, facial tics could be associated with a failure of inhibition of CSTC circuits that receive topographical projections from the orofacial regions of the (pre)motor cortex. Similarly, oculomotor CSTC circuits may be involved in eye movement tics, whereas the limbic circuit may be associated with vocalizations and comorbid obsessive-compulsive disorder (OCD) and externalizing behavior problems.[20]

The possible involvement of the basal ganglia and related cortical structures in tic disorders is supported by recent large-scale structural magnetic resonance imaging (MRI) studies involving both children and adults with TS. These have identified basal ganglia and cortical volume differences between TS patients and healthy controls.[21,22] Previously reported findings[23-25] of a lack or an inversion of normal basal ganglia volume left-right asymmetry could not be replicated.[22] However, across both children and adults with TS, volumes of the caudate nucleus were found to be significantly reduced.[22] Previously, in a study involving monozygotic twins both affected with TS, the caudate nucleus was also consistently found to be smaller in the more severely affected co-twin,[26] which points to the non-genetic nature of the identified caudate volume reduction. The most prominent feature of the recent large scale studies,[21-22] though, were strikingly different findings between children and adults with TS. In accordance with previous work,[24] adults with TS were found to have reduced lenticular nuclei volumes, which was not observed in children with TS.[22,25] Similar remarkable differences between children and adults with TS were reported with regard to cortical volumes.[21] Prefrontal and orbital frontal cortical volumes were enlarged in children with TS, but decreased in adult patients, compared to healthy controls. Finally, in a functional MRI study in which adult patients were asked to suppress their tics, prefrontal areas were found to be strongly activated.[6]

Taken together, the structural MRI studies certainly support the involvement of the basal ganglia and interconnected cortical structures, even though the identified volume differences may not necessarily be directly related to the pathophysiology of TS.[27] When considering the opposing direction of some of the neuroimaging findings in children and adults with TS, it should be remembered that adults who continue to show tics do not represent the typical course of tic disorders. Rather, the majority of TS cases have shown complete remission by the early adult years. Thus, adults with tics may form a subgroup that is unsuccessful in finding compensatory pathways to the tics. Accordingly, identified volume changes in children with TS may actually reflect such compensatory pathways, that the adults with tics would not be expected to display. In other words, volume changes may point to the plasticity of the brain in response to compensating for the presence of tics, rather than form a direct

expression of the pathogenesis of tics. Above all, these findings point to the importance of longitudinal MRI studies that could study volumes of relevant brain structures in relation to the onset, changing severity pattern, and possible remission of the tics over the course of time.[27]

Finally, in general, findings of positron-emission tomography (PET) and single-photon emission-computed tomography (SPECT) studies are also in accordance with the involvement of the basal ganglia in TS. Both reduced glucose utilization in the ventral striatum[28-30] as well as reduced blood flow in the globus pallidus and putamen[31] or the entire basal ganglia[32,33] has been reported. In addition, a recent PET study[34] compared regional cerebral glucose metabolic rates between TS patients and controls and found altered limbic-motor interactions in TS, again pointing to the relevance of the CSTC circuits. Functional connections between the motor and lateral orbitofrontal circuits were identified in both patients and controls; however, a reversal in the pattern of these interactions differentiated TS patients and controls. In TS patients, activity in the motor and lateral orbitofrontal circuits appeared to be positively correlated, whereas in healthy controls, increased activity in motor circuits appeared to be associated with relative inactivity in lateral orbitofrontal circuits.[34]

Neurochemistry and psychopharmacology

Without doubt, altered neurotransmission is involved in the pathophysiology of tics. Central neurotransmitters serve the major goal of communication within the brain, through binding to specific receptors. Several well-established research findings strongly suggest disturbances within the dopaminergic system in TS. First, dopamine receptor blocking pharmacological agents constitute the most effective tic suppressing medication.[35] Moreover, agents which increase central dopaminergic activity such as L-dopa[36] and CNS stimulants[37] may induce or exacerbate tics. Indeed, in five monozygotic twin pairs with TS, SPECT findings suggested increased dopamine receptor capacity in the caudate nucleus of the more severely affected co-twin,[38] possibly explaining the beneficial effect of dopamine antagonists in tic disorders.[35] In addition, a recent PET study demonstrated increased intrasynaptic dopamine levels in the putamen of TS patients after amphetamine challenge, which the authors linked to possible abnormal dopamine transporter regulation in TS.[39] The latter observation can be taken to suggest that in TS patients, dopamine neurons may release larger than normal amounts of the transmitter when activated, possibly resulting in loss of control of motor functions, and the emergence of tics. A recently reported animal model does tend to support a role for hyperactivation of the dopaminergic system. Transgenic mice expressing a neuropotentiating protein within a cortical-limbic subset of dopamine D1-receptor-expressing neurons were shown to demonstrate tic-like behavior.[40]

A postmortem brain study has reported that neuronal dopamine uptake sites were significantly increased in number in the caudate and the putamen, compared

to control values. This was suggested to indicate enhanced dopamine innervation within the striatum.[41] However, other postmortem brain studies have not found differences in a range of presynaptic dopaminergic markers,[42,43] and a large study of cerebrospinal fluid neurochemicals did not find altered levels of the principle dopamine metabolite homovanillic acid.[44] In addition, a PET imaging study of a presynaptic marker of dopaminergic neurons in the striatum did not find differences between TS and healthy controls.[45]

Limited additional neurochemical data are available which are suggestive of alterations of other neurotransmitter systems, including the noradrenergic system,[44] endogenous opioid peptides,[46] the serotonergic system,[43] and the glutamatergic system.[43] The report of altered glutamatergic neurochemistry in the medial globus pallidus is of interest as it is consistent with imaging studies indicating alterations in the pallidum.

Genetics

TS has a strong genetic background (recently reviewed by Pauls[47]). In a twin study, in which at least one co-twin had TS, it has been demonstrated that monozygotic twins are more often concordant for the presence of TS (53%) or any tics (77%) than dizygotic twins (8% concordant for TS, and 23% for any tics).[48] Thus, genetic factors play a profound role, but the phenotype may be variable and may not be confined to full-blown TS. Indeed, family studies have shown that family members of a proband with tics are much more likely to have tics, compared to persons in the general population.[49,50] Several independent family segregation analyses have reported that the mode of vertical transmission of TS fitted best to a mode of inheritance involving a single autosomal dominant locus with varying penetrance;[50-52] more recent studies though, have indicated that the genetic transmission is probably more complex.[53,54]

Subsequent classic multigenerational parametric linkage studies were unsuccessful in finding the presumed major TS locus, after having excluded more than 95% of the genome.[47,55] These linkage studies had been carried out under the assumption that TS is a homogeneous disorder in which a major dominant gene would be involved and that the presumed TS phenotype would include chronic motor tics. Obviously, these assumptions were challenged by the negative results of the linkage studies. In addition, there is still debate about the exact nature and range of the putative TS phenotype. According to some authors, this may include forms of OCD[56] and attention deficit/hyperactivity disorder (ADHD).[57]

Meanwhile, new strategies have been enlisted in the search for the genes involved in TS, including the investigation of a number of candidate genes and regions. Candidate genes included the dopamine[58] and adrenergic[59] receptor genes, the dopamine[60] and serotonin transporter[61] genes, the catechol-o-methyltransferase gene,[62,63] and the human leukocyte antigen locus.[64] To date, the candidate gene studies have been negative. Related studies have chosen candidate

regions based on identified chromosomal abnormalities in individual patients; however, involvement of the candidate region could either not be confirmed,[65-66] or still awaits confirmation (a novel gene by a breakpoint in 7q31;[67] and the contactin-associated protein 2 gene by the breakpoint at 7q35-7q36[68]). A recent study described a TS patient with a 18q21-q22 inversion, whereby the rearrangement was fine-mapped to within 1 Mb of a 7;18 translocation (breakpoint at 18q22) present in a previously independently described TS pedigree[69] in which that translocation co-segregated with TS and related problems in that individual's family. Interestingly, fine-mapping in the recent case report identified no structurally disrupted transcripts, but, instead, found evidence for epigenetic abnormalities. These consisted of functional dysregulations of one or more genes in the region, in the form of a significant increase in replication asynchrony in the patient compared to controls, with the inverted chromosome showing delayed replication timing across at least a 500-kb interval.[70] This finding makes the 18q22 region an interesting candidate region, and, moreover, points to a novel mechanism for neuropsychiatric pathogenesis, resulting from balanced autosomal gene rearrangements.

As a third genetic strategy, a number of non-parametric full genome scans have been carried out that used either affected sib-pairs,[71] multigenerational families,[72] or a case control design in an isolated Afrikaner population.[73,74] Although some genome regions have been implicated by these studies, only the possible involvement of the 11q23 region[73,74] has been confirmed in an independent study that involved a large French Canadian family.[75] This makes the 11q23 region probably the most interesting candidate genome area at present. A recent genome scan[76] used a more homogeneous subgroup of patients with selection based on the presence of hoarding obsessions and compulsions and found evidence that loci on 4q, 5q and 17q might be associated with this TS subtype. Only the 4q locus was near a region of potential involvement identified in the previously conducted sib-pair genome scan of unselected TS patients.[71] In conclusion, while the concept of one single major TS gene now appears untenable, considerable progress has been made in the search for the genetic background of TS. Although a number of candidate regions have been identified, most of these await confirmation. Moreover, the regions of interest will still need to be fine-mapped before anything specific can be said about TS genetics at a gene level.

Notwithstanding the clear involvement of genetic factors in TS, the non-100% concordance rates for TS in monozygotic twins[48] also point to the involvement of non-genetic elements. Indeed, a number of environmental factors have been identified. These include adverse prenatal events, as evidenced by the lower birth weight in the more severely affected co-twin of monozygotic twin pairs with TS[77] and a reported association of maternal stress and severe nausea and vomiting during pregnancy with later tic severity in the off-spring.[78] Also, stressful life events have been linked to fluctuations in tic severity to some extent.[79] Finally, there has been quite some interest recently in the possible adverse consequences of certain infections, both with respect to tic induction and

tic exacerbations. We will review this possible relationship in the following sections.

Possible role of infections in tic disorders

In the mid-1990s, several case studies entered the literature in which children were described who suddenly demonstrated severe forms of tics and obsessive-compulsive symptoms, which the authors linked to signs and symptoms of streptococcal infections.[80-82] Confronted with a failure of conventional treatment approaches, authors described the successful application of immune-based treatments in some of these cases, consisting of therapeutic plasma exchange,[80-82] intravenously administered immunoglobulins,[82-84] or simple use of antibiotics[82] to treat the infectious process. Such treatment modalities had not been previously employed in child psychiatry. In some cases, streptococcal reinfections were associated with the reinduction of neuropsychiatric symptoms.[85] Also, there was the notion that patients with Sydenham's chorea, which has a well-established link to group A streptococcal infections, often show behavioral symptoms, including obsessions, compulsions, and emotional lability.[86] Finally, a study had suggested the presence of autoantibodies reacting with brain tissue in patients with tics and/or OCD.[87] All in all, these observations led researchers at the National Institute of Mental Health to formulate clinical criteria for a putative subgroup of children with OCD or TS in whom symptom exacerbations were abrupt, dramatic, and temporally related to group A beta-hemolytic streptococcal infections. The subgroup was designated by the euphonious acronym PANDAS (pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections).[85,88] PANDAS inclusion criteria were outlined, that require the presence of OCD and/or tic disorder, pre-pubertal symptom onset, sudden onset or episodic course of symptoms, temporal association between streptococcal infections and neuropsychiatric symptom exacerbations, and associated neurological abnormalities.

The concept of PANDAS strongly centers on Sydenham's chorea as a putative disease model, including the suggested pathogenic role of autoantibodies that cross-react with brain antigens as a hypothesized consequence of structural homology with streptococcal antigens.[89] Introduction of the concept of PANDAS has certainly done much to stimulate research and clinical interest in the potential relevance of immune factors in tic and related disorders. Concerns regarding the validity of PANDAS have been raised, however.[90-93] These include criticism about the vagueness[92,93] of some of the criteria for PANDAS. For example, when should an exacerbation be considered abrupt? Or what time frame exactly constitutes a temporal relationship between group A beta-hemolytic streptococcal infections and symptom exacerbations? Also, concerns regarding diagnostic criteria for a streptococcal infection have been raised.[90,91] Should a positive throat swab, without raised antistreptococcal antibody levels or symptoms suggestive of pharyngitis[94] really be considered a streptococcal infection? As an example, in a recent study,[95]

investigators claimed to have prospectively identified a number of PANDAS cases, without, however, having obtained serological evidence in the majority of putative cases.

In addition to the questions regarding criteria, we have three more fundamental concerns regarding the concept of PANDAS. First, in our opinion, it is far too early to call tic and related disorders "autoimmune", even when and if there is an established association with infections. As will be discussed in a later section, proving the involvement of autoimmunity in a neuropsychiatric disorder is a formidable challenge and requires much more than showing a connection with infection.

Second, studies on PANDAS bear the very real risk of circular reasoning: what you include, is what you find. In our opinion, research that attempts to define the extent of immune involvement in neuropsychiatric disorders should preclude the a priori selection of cases that, by definition, demonstrate a temporal relationship with streptococcal infections. Moreover, in a previous review,[92] we pointed to the fact that, so far, no laboratory markers for immune dysregulation have been identified in PANDAS cases that have not also been described in unselected tic disorder patients. Therefore, we strongly argue for the use of unselected tic disorder patients in future studies on the extent of involvement of immune factors in tic disorders. This is not to reject the possibility that careful longitudinal studies of extreme phenotypes (eg those patients with the most elevated antibody titers or those with the most marked exacerbations) might also provide a useful approach.

A third concern is that the required association with streptococcal infections in PANDAS seriously hampers unbiased investigation of other possibly involved infections in tic disorders. As an example, Allen[82] described case studies associated with viral infections. In addition, two TS cases associated with mycoplasma infections have recently been described.[96] Also, in a 6 month longitudinal study with unselected tic disorder patients, our group recently identified a highly significant association between the occurrence of a common cold and a subsequent exacerbation in tic severity in pediatric patients 4 weeks later, after symptoms of the cold had long disappeared. [97] The involvement of streptococcal infections in our study could be ruled out, given the finding of negative throat swabs and absent rise of streptococcal antibody levels in our study participants. Previously, Cardona[98] had reported a more frequent history of upper respiratory tract infections in tic disorder patients than in healthy controls subjects. Also of interest is a recent study that pointed to the importance of common colds at the time of onset of OCD and tic symptoms.[99] Thus, infections other than streptococcal infections may well be involved in tic disorders; these may include relatively common viral infections.

It is remarkable that four studies that did not preselect tic disorder according to PANDAS criteria probably found the best available evidence for an association of tic disorders with streptococcal infections,[98,100-102] even when the exact nature of this possible association remains unclear. In essence, these studies reported markedly increased serum levels of antistreptococcal antibodies in a cross-sectional single time point design. Interestingly, Cardona[98] found that levels of antistreptolysin O correlated with severity of the tics, whereas the recent study

of Church and coworkers[102] identified an intriguing association between raised antistreptolysin O titers and presence of antibasal ganglia antibodies, as assessed by Western blotting and indirect immunofluorescence in unselected TS subjects. The cross-sectional design makes these findings extremely hard to interpret, especially since antibody levels in these subjects were not assessed at the time of the first appearance of their tics, nor at a time of symptom exacerbation, but rather at an arbitrary point in time. Are patients with tics more likely to encounter streptococcal infections? Or does their immune system differently respond to streptococci, which may include a more prolonged humoral response? We really need longitudinal data to answer these and other questions. In contrast, several US studies failed to find increased single time point levels of antistreptococcal antibodies in tic disorder subjects.[103-106] Thus, even though some data may support an association between streptococcal infections and tic disorders, at present the association does not appear to be universally present, but may be confined to certain geographical areas. The potential importance of the topic lies partially in the possible relevance to treatment: the use of antibiotics to treat the underlying infections has received limited attention so far,[94] but is certainly a topic that deserves further consideration.

In conclusion, some evidence is available about a possible association of streptococcal infections with tic disorders. Little can be said, however, about the nature of this association. Moreover, there is also some indication about the possible involvement of non-streptococcal infections, including common viral infections, in exacerbations of tic disorders. With much interest we await the outcome of the ongoing longitudinal studies on this topic.

B lymphocyte surface marker D8/17

Besides a possible association of tic disorders with infections, a number of laboratory findings have been reported in tic disorders that may support the involvement of immune dysregulation. We will first review a line of research which focuses on overexpression of a B lymphocyte surface marker, designated D8/17, a putative marker of susceptibility to rheumatic fever, and then summarize the findings regarding assessment of autoantibodies against neuronal tissue. The monoclonal antibody against the D8/17-epitope is a mouse monoclonal IgM antibody that was originally prepared from fusions of spleen cells from mice that had repeatedly been immunized with isolated human B lymphocytes obtained from rheumatic fever or rheumatic heart disease patients.[107] D8/17-specific monoclonal antibody has been reported to bind with a small percentage of B lymphocytes in normal controls (averaging 5-7%), but in rheumatic fever the percentage of D8/17 positive B lymphocytes was found to be much higher (33.5% in average).[107] It was proposed that an individual could be classified as D8/17-positive when the percentage of D8/17-positive B lymphocytes exceeded the mean plus one standard deviation of that of healthy controls (that is, 12%). An individual's D8/17-positivity has been reported to be a susceptibility marker for rheumatic fever,

a well-known complication of infections with group A beta-hemolytic streptococci, with 60%-100% of rheumatic fever subjects reported to be D8/17-positive.[108]

In 1997, the first two reports on D8/17 in tic disorders appeared in the literature.[88,109] Results of these studies were quite remarkable: when subjects were categorized as either D8/17-positive or D8/17-negative according to the previously established cutoff of more than 12% D8/17-positive B lymphocytes, D8/17-positivity appeared almost diagnostic for the presence of a tic disorder or OCD. In one study,[109] 100% of patients (either having childhood-onset OCD or TS) appeared to be D8/17-positive versus less than 5% of healthy controls, whereas the other study[88] reported 85% of patients fulfilling PANDAS criteria to be D8/17-positive versus 17% of healthy controls. Subsequently, an association of D8/17-positivity was also reported with other psychiatric disorders, including autistic disorder,[110] trichotillomania,[111] and anorexia nervosa.[112]

Although these D8/17 findings are intriguing, the method with which they were obtained has serious flaws. In all studies mentioned so far, D8/17 expression on B cells had been assessed by indirect immunofluorescence whereby individual B cells were visually categorized as either D8/17-positive or D8/17-negative and counted manually by fluorescence microscopy. Three studies have used the more objective method of flow cytometry[113-115] and also found group differences between patients with a tic disorder and/or OCD and healthy controls. However, our group made use of a control IgM monoclonal antibody in addition to the antibody directed against the D8/17 epitope.[114] For this purpose, we used MOC32, an IgM monoclonal antibody that is directed against a neuroendocrine antigen of epithelial origin of small cell lung cancer cells. Upon re-examination of our data, it now appears likely that we did not detect D8/17 overexpression on B cells in tic disorder patients compared to healthy controls, but, rather, increased expression of receptors for the constant parts of IgM molecules (Fc-μ) on B cells. Thus, this appears to suggest that tic disorder patients do not express a specific susceptibility marker for experiencing autoimmune sequelae in the aftermath of streptococcal infections. Instead, the evidence may be indicative of a generalized increased immune activity.

Whatever is measured when assessing D8/17 B cell overexpression, we and other centers encountered major reproducibility problems in subsequent studies, which were often not published. The D8/17-specific antibody appeared to gradually lose its patient-control discriminating abilities in the course of months (unpublished observations from our laboratory). In addition, there appeared to be major differences between different antibody batches, an experience we share with others in the field.[116] At present, the molecular nature of the D8/17 epitope is unknown. Many centers have stopped using this antibody, given the failure to replicate group differences.[117] Finally, a recently published study failed to find an association between D8/17-positivity and the presence of tics or OCD in a community sample.[118] Thus, a line of research that seemed quite promising at the outset, now appears to be floundering due to lack of progress in characterizing the presumed susceptibility marker. It is possible that previous positive reports were due to an unspecific increase of the number of Fc-μ receptors on B cells, a possibility that certainly deserves further study.

Antineuronal autoantibodies

The presence of autoantibodies reacting with parts of the brain that are thought to be involved in tic disorders, is potentially a strong line of evidence in favor of the autoimmune hypothesis of tic disorders. In a previous review, we summarized the work carried out in this area.[92] In short, two studies assessed autoantibody binding to human caudate tissue with an indirect immunofluorescence technique in patients with tics and/or OCD,[87,109] as previously applied in patients with Sydenham's chorea.[119] Results in both studies were similar: positive staining was reported in 44%-50% of tic disorder patients versus 21%-24% of healthy controls. Subsequent studies, using enzyme-linked immunosorbent assays against either an immortalized neuronal cell line,[120,121] human basal ganglia,[104] or rat brain,[103] in general, confirmed the increased levels of serum antineuronal antibodies in TS patients.

Some support for the direct involvement of antineuronal autoantibodies in the disease process stems from two different research findings. First, intravenously administered immunoglobulins and therapeutic plasma exchange were reported to be highly effective treatments in cases fulfilling PANDAS criteria.[122] These treatment modalities are thought to block or remove the antineuronal autoantibodies, respectively. As yet, this finding still awaits confirmation. A second pillar possibly supporting a pathogenic role of antineuronal antibodies is formed by two studies in which animal models were developed to study whether serum or purified IgG from patients with TS can induce tic-like behavior in rats.[123,124] In these studies, serum or IgG was microinfused through cannulas placed in regions of the neostriatum known to induce stereotypies, after which the rats were observed for development of movements or utterances. Hallett et al.[123] infused dilute serum from five TS patients with high antibody titers against human neuroblastoma bilaterally into the ventral striatal region of the rat. Results showed a significant increase in tic-like behaviors (eg, licks and forepaw shakes) and episodic utterances in rats infused with TS sera, which was not observed when sera from healthy controls were microinfused. Taylor et al.[124] infused serum from 12 TS patients with high antibody titers against rat striatum bilaterally into a different brain area, the ventrolateral striatal region. Results showed a significant increase of high titer-induced oral stereotypies over a 5 days' period of observation. These intriguing results were not confirmed in a recent study in which Loiselle and coworkers microinfused serum from five TS children with high antibody titers against human postmortem putamen bilaterally into the ventral and ventrolateral striatum. In that study, no rat was reported to develop any audible abnormality and there was no significant increase in stereotypic behaviors.[93] At our center, we also infused TS sera into rat brains at the same coordinates used in the Hallett and Taylor protocols, but did not identify any differences in tic-like behavior compared to rats that had been microinfused with sera from healthy controls (unpublished results). We have no explanation for

these conflicting results across different centers. Future studies might profit from across-center sharing of reference sera that had been previously demonstrated to induce tics.

It should be noted that, as yet, no single neuronal antigenic structure has been identified as target for the putative antineuronal antibodies. Only a limited number of studies have aimed to identify separate neuronal antigens, by using Western blot techniques. Most of these studies detected candidate antigenic structures, derived from either brain tissue or a neuroblastoma cell line, with an apparent molecular weight of 60 and/or 83 kD.[102,104,121,125] In another Western blot study, our group recently detected, in line with existing studies, more frequent seroreactivity in tic disorder patients against a 60 kD protein band from a neuronal cell line compared to healthy controls, patients with autistic disorder, and patients with OCD.[126] Subsequent sequence analysis identified this 60 kD band as the human 60 kD heat shock protein. This protein is by no means confined to neuronal tissue; on the contrary, the human 60 kD heat shock protein is considered to be ubiquitous.[127]

Although involvement of specific neuronal antigens cannot be ruled out, the results of our study indicate that autoantibody binding to tissue of neuronal origin does not necessarily have to be confined to exclusively neuronal epitopes. This may as well explain the findings of antineuronal antibodies in sera of healthy controls, as reported in earlier studies.[87,109] Though we could not directly link the presence of autoantibodies to the pathogenesis of tic disorders, our findings are still likely to support the involvement of immune factors in tic disorders. Inappropriate reactivity to heat shock proteins in humans may be understood, given the high degree of antigenic homology between microbial and human hsp60, which may lead to harmful cross-reactivity with human structures.[128] There is well-established evidence that inappropriate reactivity to heat shock proteins is involved in autoimmune disorders,[129] including rheumatic autoimmune diseases,[130,131] severe coronary heart disease, and carotid atherosclerosis.[132] Also, increased anti-hsp60 binding may be due to frequently encountered infections.

In conclusion, the assessment of serum autoantibodies has appeared to be a fruitful research area in tic disorders. However, this area is very much a work-in-progress, especially with regard to characterizing antigenic structures at a molecular level and elucidating the pathogenic relevance of the antibody-antigen interactions in animal models.

Conclusions

Considerable progress has been made over the last decade with regard to the neurobiology of TS. Convincing evidence indicates that the basal ganglia and related cortico-striato-thalamo-cortical circuits are likely to be involved in both the tics and related behavioral abnormalities. Some intriguing, though quite preliminary neurochemical data are available that appear to indicate differences in specific neurotransmitter systems. Much progress has also been made in the field

of genetics. While independent twin and family studies had indicated the genetic background of TS and related disorders some time ago, now a number of genetic linkage and association studies have provided the first candidate loci on the human genome.

Apart from genetic findings, a number of environmental factors have been implicated in the pathogenesis and pathophysiology of TS, most notably adverse perinatal events and infections. Most research in this latter area has used Sydenham's chorea as a medical model, and thus has largely focused on the role of streptococcal infections and assessment of cross-reacting antibodies against human brain regions. Evidence for the relevance of this model for TS is still scarce, however, and is entirely based on cross-sectional studies. However, nonspecific immune activation may play a more profound role. This hypothesis is based on our findings of possibly increased receptors for the Fc-fragment of IgM on B lymphocytes, the notion of a temporal association of common viral upper respiratory infections with exacerbations of tic severity, and increased anti-hsp60 autoantibody binding in tic disorders. These findings all point to a rather general activation of the immune system that may be associated with tic disorders. Thus, even though the precise mechanism is largely unknown, at present, the available evidence does indeed seem to suggest that immune factors do appear to matter in at least some individuals with tic disorders. Ongoing large-scale longitudinal studies will have to provide definite answers to this intriguing topic.

Several lines of evidence suggest that TS is likely to be a heterogeneous disorder, meaning that different pathways may lead to the disorder. Thus, clinically relevant subgroups may emerge. For example, neuroimaging studies suggest that adults who continue to demonstrate tics may have unique neuroimaging findings which are often opposite from what has been found in pediatric patients. Neurotransmitter abnormalities may give clues for subgroups in which a certain neurotransmitter system may or may not be involved. To illustrate this, dopamine antagonists do not appear to have effect on tics in a significant minority of patients. Similarly, autoimmunity may be involved in only a subgroup of patients. Future genetic linkage and association studies may give better results when homogeneous subgroups are used, based on either clinical features or biological markers. Also, combining different research tools, eg neuroimaging with immune factors, may prove fruitful. Giedd et al.[81] described an interesting case study in which enlargement of basal ganglia volumes decreased dramatically in response to therapeutic plasma exchange. Peterson and colleagues[105] studied the relationships between antistreptococcal antibody titers and basal ganglia volumes in a cross-sectional design. Interestingly, higher antibody titers in subjects with ADHD or OCD were associated with larger volumes of the putamen and globus pallidus nuclei; surprisingly, higher titers were not seen in tic disorder patients. In another example of combining research approaches, the National Institute of Mental Health group assessed first-degree relatives of 54 children fulfilling criteria for PANDAS for the presence of a tic disorder.[133] Results were remarkably similar to those of family studies using non-PANDAS tic disorder probands. Integrating neuroimaging, neurochemistry,

genetics, and neuroimmunology may prove to be an especially powerful approach. Ultimately, however, identifying the involvement of major genes will allow for more definite studies of gene-environment interactions, both through study of animal models and of high-risk children known to be affected by such genes.

References

1. American Psychiatric Association (2000) Diagnostic and Statistical Manual of Mental Disorders, 4th edition, text revision (DSM-IV-TR). American Psychiatric Association, Washington, DC

2 . Khalifa N. and von Knorring A. L. (2003) Prevalence of tic disorders and Tourette syndrome in a Swedish school population. *Dev. Med. Child Neurol*. 45: 315-319

3. Coffey B. J., Biederman J., Geller D. A., Spencer T., Park K. S., Shapiro S. J. et al. (2000) The course of Tourette's disorder: A literature review. *Harv. Rev. Psychiatry* 8: 192-198

4. Santangelo S. L., Pauls D. L., Lavori P. W., Goldstein J. M., Faraone S. V., and Tsuang M. T. (1996) Assessing risk for the Tourette spectrum of disorders among first-degree relatives of probands with Tourette syndrome. *Am. J. Med. Genet*. 67: 107-116

5. Kushner H. I. (1999) A cursing brain? The histories of Tourette Syndrome. Harvard University Press, London

6. Peterson B. S., Skudlarski P., Anderson A. W., Zhang H., Gatenby J. C., Lacadie C. M. et al. (1998) A functional magnetic resonance imaging study of tic suppression in Tourette syndrome. *Arch. Gen. Psychiatry* 55: 326-333

7. Miguel E. C., do Rosario-Campos M. C., Prado H. S., do Valle R., Rauch S. L., Coffey B. J. et al.(2000) Sensory phenomena in obsessive-compulsive disorder and Tourette's disorder*. J. Clin. Psychiatry* 61: 150-156

8. Peterson B. S. and Leckman J. F. (1998) The temporal dynamics of tics in Gilles de la Tourette syndrome. *Biol. Psychiatry* 44: 1337-1348

9. Bruun R. D. and Budman C. L. (1997) The course and prognosis of Tourette syndrome. *Neurol. Clin*. 15: 291-298

10. Lin H., Yeh C. B., Peterson B. S., Scahill L., Grantz H., Findley D. B. et al. (2002) Assessment of symptom exacerbations in a longitudinal study of children with Tourette's syndrome or obsessive-compulsive disorder. *J. Am. Acad. Child Adolesc. Psychiatry* 41: 1070-1077

11. Leckman J. F., Zhang H., Vitale A., Lahnin F., Lynch K., Bondi C. et al. (1998) Course of tic severity in Tourette syndrome: the first two decades. *Pediatrics* 102: 14-19

12. Tanner C. M. and Goldman S. M. (1997) Epidemiology of Tourette syndrome. *Neurol. Clin.* 15: 395-402

13. Sukhodolsky D. G., Scahill L., Zhang H., Peterson B. S., King R. A., Lombroso P. J. et al. (2003) Disruptive behavior in children with Tourette's syndrome: association with ADHD comorbidity, tic severity, and functional impairment. *J. Am. Acad. Child Adolesc. Psychiatry* 42: 98-105

14. Leckman J. F., Pauls D. L., Zhang H., Rosario-Campos M. C., Katsovich L., Kidd K. K. et al. (2003) Obsessive-compulsive symptom dimensions in affected sibling pairs diagnosed with Gilles de la Tourette syndrome. *Am. J. Med. Genet*. 116: 60-68

15. Carter A. S., O'Donnell D. A., Schultz R. T., Scahill L., Leckman J. F., and Pauls D. L. (2000) Social and emotional adjustment in children affected with Gilles de la Tourette's syndrome: associations with ADHD and family functioning. Attention Deficit Hyperactivity Disorder. *J. Child Psychol. Psychiatry* 41: 215-223

16. Abbruzzese G. and Berardelli A. (2003) Sensorimotor integration in movement disorders. *Mov. Disord*. 18: 231-240

17. Wichmann T. and DeLong M. R. (2003) Functional neuroanatomy of the basal ganglia in Parkinson's disease. *Adv. Neurol*. 91: 9-18

18. Ward C. D. (2003) Neuropsychiatric interpretations of postencephalitic movement disorders. *Mov. Disord*. 18: 623-630

19. Leckman J. F., Peterson B. S., Anderson G. M., Arnsten A. F., Pauls D. L., and Cohen D. J. (1997) Pathogenesis of Tourette's syndrome. *J. Child Psychol. Psychiatry* 38: 119-142

20. Mink J. W. (2001) Neurobiology of basal ganglia circuits in Tourette syndrome: faulty inhibition of unwanted motor patterns? *Adv. Neurol*. 85: 113-122

21. Peterson B. S., Staib L., Scahill L., Zhang H., Anderson C., Leckman J. F. et al. (2001) Regional brain and ventricular volumes in Tourette syndrome. *Arch. Gen. Psychiatry* 58: 427-440

22. Peterson B. S., Thomas P., Kane M. J., Scahill L., Zhang H., Bronen R. et al. (2003) Basal Ganglia volumes in patients with Gilles de la Tourette syndrome. *Arch. Gen. Psychiatry* 60: 415-424

23. Moriarty J., Varma A. R., Stevens J., Fish M., Trimble M. R., and Robertson M. M. (1997) A volumetric MRI study of Gilles de la Tourette's syndrome. *Neurology* 49: 410-415

24. Peterson B., Riddle M. A., Cohen D. J., Katz L. D., Smith J. C., Hardin M. T. et al. (1993) Reduced basal ganglia volumes in Tourette's syndrome using three-dimensional reconstruction techniques from magnetic resonance images [see comments]. *Neurology* 43: 941-949

25. Singer H. S., Reiss A. L., Brown J. E., Aylward E. H., Shih B., Chee E. et al. (1993) Volumetric MRI changes in basal ganglia of children with Tourette's syndrome. *Neurology* 43: 950-956

26. Hyde T. M., Stacey M. E., Coppola R., Handel S. F., Rickler K. C., and Weinberger D. R. (1995) Cerebral morphometric abnormalities in Tourette's syndrome: a quantitative MRI study of monozygotic twins. *Neurology* 45: 1176-1182

27. Gerard E. and Peterson B. S. (2003) Developmental processes and brain imaging studies in Tourette syndrome. *J. Psychosom. Res*. 55: 13-22

28. Stoetter B., Braun A. R., Randolph C., Gernert J., Carson R. E., Herscovitch P. et al. (1992) Functional neuroanatomy of Tourette syndrome. Limbic-motor interactions studied with FDG PET. *Adv. Neurol*. 58: 213-226

29. Braun A. R., Randolph C., Stoetter B., Mohr E., Cox C., Vladar K. et al. (1995) The functional neuroanatomy of Tourette's syndrome: an FDG-PET Study. II: Relationships between regional cerebral metabolism and associated behavioral and cognitive features of the illness. *Neuropsychopharmacology* 13: 151-168

30. Chase T. N., Geoffrey V., Gillespie M., and Burrows G. H. (1986) Structural and functional studies of Gilles de la Tourette syndrome. *Rev. Neurol*. (Paris) 142: 851-855

31. Riddle M. A., Rasmusson A. M., Woods S. W., and Hoffer P. B. (1992) SPECT imaging of cerebral blood flow in Tourette syndrome. *Adv. Neurol*. 58: 207-211

32. Moriarty J., Costa D. C., Schmitz B., Trimble M. R., Ell P. J., and Robertson M. M. (1995) Brain perfusion abnormalities in Gilles de la Tourette's syndrome. *Br. J. Psychiatry* 167: 249-254

33. Klieger P. S., Fett K. A., Dimitsopulos T., and Karlan R. (1997) Asymmetry of basal ganglia perfusion in Tourette's syndrome shown by technetium-99m-HMPAO SPECT. *J. Nucl. Med*. 38: 188-191

34. Jeffries K. J., Schooler C., Schoenbach C., Herscovitch P., Chase T. N., and Braun A. R. (2002) The functional neuroanatomy of Tourette's syndrome: an FDG PET study III: functional coupling of regional cerebral metabolic rates. *Neuropsychopharmacology* 27: 92-104

35. Bruggeman R., van der Linden C., Buitelaar J. K., Gericke G. S., Hawkridge S. M., and Temlett J. A. (2001) Risperidone versus pimozide in Tourette's disorder: a comparative double-blind parallel-group study. *J. Clin. Psychiatry* 62: 50-56

36. Klempel K. (1974) Gilles de la Tourette's symptoms induced by L-dopa. S. *Afr. Med. J*. 48: 1379-1380

37. Robertson M. M. and Eapen V. (1992) Pharmacologic controversy of CNS stimulants in Gilles de la Tourette's syndrome. *Clin. Neuropharmacol*. 15: 408-425

38. Wolf S. S., Jones D. W., Knable M. B., Gorey J. G., Lee K. S., Hyde T. M. et al. (1996) Tourette syndrome: prediction of phenotypic variation in monozygotic twins by caudate nucleus D2 receptor binding. *Science* 273: 1225-1227

39. Singer H. S., Szymanski S., Giuliano J., Yokoi F., Dogan A. S., Brasic J. R. et al. (2002) Elevated intrasynaptic dopamine release in Tourette's syndrome measured by PET. *Am. J. Psychiatry* 159: 1329-1336

40. Nordstrom E. J. and Burton F. H. (2002) A transgenic model of comorbid Tourette's syndrome and obsessive-compulsive disorder circuitry. *Mol. Psychiatry* 7: 617-625

41. Singer H. S., Hahn I. H., and Moran T. H. (1991) Abnormal dopamine uptake sites in postmortem striatum from patients with Tourette's syndrome. *Ann. Neurol*. 30: 558-562

42. Anderson G. M., Pollak E. S., Chatterjee D., Leckman J. F., Riddle M. A., and Cohen D. J. (1992) Postmortem analysis of subcortical monoamines and amino acids in Tourette syndrome. *Adv. Neurol*. 58: 123-133

43. Anderson G. M., Pollak E. S., Chatterjee D., Leckman J. F., Riddle M. A., and Cohen D. J. (1992) Brain monoamines and amino acids in Gilles de la Tourette's syndrome: a preliminary study of subcortical regions. *Arch. Gen. Psychiatry* 49, 584-586.

44. Leckman J. F., Goodman W. K., Anderson G. M., Riddle M. A., Chappell P. B., McSwiggan-Hardin M. T. et al. (1995) Cerebrospinal fluid biogenic amines in obsessive compulsive disorder, Tourette's syndrome, and healthy controls. *Neuropsychopharmacology* 12: 73-86

45. Meyer P., Bohnen N. I., Minoshima S., Koeppe R. A., Wernette K., Kilbourn M. R. et al. (1999) Striatal presynaptic monoaminergic vesicles are not increased in Tourette's syndrome. *Neurology* 53: 371-374

46. van Wattum P. J., Chappell P. B., Zelterman D., Scahill L. D., and Leckman J. F. (2000) Patterns of response to acute naloxone infusion in Tourette's syndrome. *Mov Disord*. 15: 1252-1254

47. Pauls D. L. (2003) An update on the genetics of Gilles de la Tourette syndrome. *J. Psychosom. Res*. 55: 7-12

48. Price R. A., Kidd K. K., Cohen D. J., Pauls D. L., and Leckman J. F. (1985) A twin study of Tourette syndrome. *Arch. Gen. Psychiatry* 42: 815-820

49. Hebebrand J., Klug B., Fimmers R., Seuchter S. A., Wettke-Schafer R., Deget F. et al. (1997) Rates for tic disorders and obsessive compulsive symptomatology in families of children and adolescents with Gilles de la Tourette syndrome. *J. Psychiatr. Res*. 31: 519-530

50. Eapen V., Pauls D. L., and Robertson M. M. (1993) Evidence for autosomal dominant transmission in Tourette's syndrome. United Kingdom cohort study. *Br. J. Psychiatry* 162: 593-596

51. Devor E. J. (1984) Complex segregation analysis of Gilles de la Tourette syndrome: further evidence for a major locus mode of transmission. *Am. J. Hum. Genet.* 36: 704-709

52. Pauls D. L. and Leckman J. F. (1986) The inheritance of Gilles de la Tourette's syndrome and associated behaviors. Evidence for autosomal dominant transmission. *N. Engl. J. Med*. 315: 993-997

53. Walkup J. T., LaBuda M. C., Singer H. S., Brown J., Riddle M. A., and Hurko O. (1996) Family study and segregation analysis of Tourette syndrome: evidence for a mixed model of inheritance. *Am. J. Hum. Genet*. 59: 684-693

54. Seuchter S. A., Hebebrand J., Klug B., Knapp M., Lehmkuhl G., Poustka F. et al. (2000) Complex segregation analysis of families ascertained through Gilles de la Tourette syndrome. *Genet. Epidemiol.* 18: 33-47

55. Pakstis A. J., Heutink P., Pauls D. L., Kurlan R., Van De Wetering B. J., Leckman J. F. et al. (1991) Progress in the search for genetic linkage with Tourette syndrome: an exclusion map covering more than 50% of the autosomal genome. *Am. J. Hum. Genet*. 48: 281-294

56. Santangelo S. L., Pauls D. L., Goldstein J. M., Faraone S. V., Tsuang M. T., and Leckman J. F. (1994) Tourette's syndrome: what are the influences of gender and comorbid obsessive-compulsive disorder? *J. Am. Acad. Child Adolesc. Psychiatry* 33: 795-804

57. Knell E. R. and Comings D. E. (1993) Tourette's syndrome and attention-deficit hyperactivity disorder: evidence for a genetic relationship. *J. Clin. Psychiatry* 54: 331-337

58. Brett P. M., Curtis D., Robertson M. M., and Gurling H. M. (1995) The genetic susceptibility to Gilles de la Tourette syndrome in a large multiple affected British kindred: linkage analysis excludes a role for the genes coding for dopamine D1, D2, D3, D4, D5

receptors, dopamine beta hydroxylase, tyrosinase, and tyrosine hydroxylase. *Biol. Psychiatry* 37: 533-540

59. Xu C., Ozbay F., Wigg K., Shulman R., Tahir E., Yazgan Y. et al (2003) Evaluation of the genes for the adrenergic receptors alpha2A and alpha1C and Gilles de la Tourette Syndrome. *Am. J. Med. Genet*. 119B: 54-59

60. Vandenbergh D. J., Thompson M. D., Cook E. H., Bendahhou E., Nguyen T., Krasowski M. D. et al. (2000) Human dopamine transporter gene: coding region conservation among normal, Tourette's disorder, alcohol dependence and attention-deficit hyperactivity disorder populations. *Mol. Psychiatry* 5: 283-292

61. Gelernter J., Rao P. A., Pauls D. L., Hamblin M. W., Sibley D. R., and Kidd K. K. (1995) Assignment of the 5HT7 receptor gene (HTR7) to chromosome 10q and exclusion of genetic linkage with Tourette syndrome. *Genomics* 26: 207-209

62. Barr C. L., Wigg K. G., and Sandor P. (1999) Catechol-O-methyltransferase and Gilles de la Tourette syndrome. *Mol. Psych*iatry 4: 492-495

63. Cavallini M. C., Di Bella D., Catalano M., and Bellodi L. (2000) An association study between 5-HTTLPR polymorphism, COMT polymorphism, and Tourette's syndrome. *Psychiatry Res*. 97: 93-100

64. Schoenian S., Konig I., Oertel W., Remschmidt H., Ziegler A., Hebebrand J. et al. (2003) HLA-DRB genotyping in Gilles de la Tourette patients and their parents. *Am. J. Med. Genet*. 119B: 60-64

65. Brett P. M., Curtis D., Robertson M. M., Dahlitz M., and Gurling H. M. (1996) Linkage analysis and exclusion of regions of chromosomes 3 and 8 in Gilles de la Tourette syndrome following the identification of a balanced reciprocal translocation 46 XY, t(3:8)(p21.3 q24.1) in a case of Tourette syndrome. *Psychiatr. Genet.* 6: 99-105

66. Matsumoto N., David D. E., Johnson E. W., Konecki D., Burmester J. K., Ledbetter D. H. et al. (2000) Breakpoint sequences of an 1;8 translocation in a family with Gilles de la Tourette syndrome. *Eur. J. Hum. Genet*. 8: 875-883

67. Petek E., Windpassinger C., Vincent J. B., Cheung J., Boright A. P., Scherer S. W. et al. (2001) Disruption of a novel gene (IMMP2L) by a breakpoint in 7q31 associated with Tourette syndrome. *Am. J. Hum. Genet*. 68: 848-858

68. Verkerk A. J., Mathews C. A., Joosse M., Eussen B. H., Heutink P., and Oostra B. A. (2003) CNTNAP2 is disrupted in a family with Gilles de la Tourette syndrome and obsessive compulsive disorder. Genomics 82: 1-9

69. Boghosian-Sell L., Comings D. E., and Overhauser J. (1996) Tourette syndrome in a pedigree with a 7;18 translocation: identification of a YAC spanning the translocation breakpoint at 18q22.3. *Am. J. Hum. Genet*. 59: 999-1005

70. State M. W., Greally J. M., Cuker A., Bowers P. N., Henegariu O., Morgan T. M. et al. (2003) Epigenetic abnormalities associated with a chromosome 18(q21-q22) inversion and a Gilles de la Tourette syndrome phenotype. *Proc. Natl. Acad. Sci*. U. S. A 100: 4684-4689

71. The Tourette Syndrome Association International Consortium for Genetics. (1999) A complete genome screen in sib pairs affected by Gilles de la Tourette syndrome. *Am. J. Hum. Genet*. 65: 1428-1436

72. Barr C. L., Wigg K. G., Pakstis A. J., Kurlan R., Pauls D., Kidd K. K. et al. (1999) Genome scan for linkage to Gilles de la Tourette syndrome. *Am. J. Med. Genet.* 88: 437-445

73. Simonic I., Nyholt D. R., Gericke G. S., Gordon D., Matsumoto N., Ledbetter D. H. et al. (2001) Further evidence for linkage of Gilles de la Tourette syndrome (GTS) susceptibility loci on chromosomes 2p11, 8q22 and 11q23-24 in South African Afrikaners. *Am. J. Med. Genet*. 105: 163-167

74. Simonic I., Gericke G. S., Ott J., and Weber J. L. (1998) Identification of genetic markers associated with Gilles de la Tourette syndrome in an Afrikaner population. *Am. J. Hum. Genet*. 63: 839-846

75. Merette C., Brassard A., Potvin A., Bouvier H., Rousseau F., Emond C. et al. (2000) Significant linkage for Tourette syndrome in a large French Canadian family. *Am. J. Hum. Genet*. 67: 1008-1013

76. Zhang H., Leckman J. F., Pauls D. L., Tsai C. P., Kidd K. K., and Campos M. R. (2002) Genomewide scan of hoarding in sib pairs in which both sibs have Gilles de la Tourette syndrome. *Am. J. Hum. Genet*. 70: 896-904

77. Hyde T. M., Aaronson B. A., Randolph C., Rickler K. C., and Weinberger D. R. (1992) Relationship of birth weight to the phenotypic expression of Gilles de la Tourette's syndrome in monozygotic twins. *Neurology* 42: 652-658

78. Leckman J. F., Dolnansky E. S., Hardin M. T., Clubb M., Walkup J. T., Stevenson J. et al. (1990) Perinatal factors in the expression of Tourette's syndrome: an exploratory study. *J. Am. Acad. Child Adolesc. Psychiatry* 29: 220-226

79. Findley D. B., Leckman J. F., Katsovich L., Lin H., Zhang H., Grantz H. et al. (2003) Development of the Yale Children's Global Stress Index (YCGSI) and its application in children and adolescents ith Tourette's syndrome and obsessive-compulsive disorder. *J. Am. Acad. Child Adolesc. Psychiatry* 42: 450-457

80. Tucker D. M., Leckman J. F., Scahill L., Wilf G. E., LaCamera R., Cardona L. et al. (1996) A putative poststreptococcal case of OCD with chronic tic disorder, not otherwise specified [clinical conference] [see comments]. *J. Am. Acad. Child Adolesc. Psychiatry* 35: 1684-1691

81. Giedd J. N., Rapoport J. L., Leonard H. L., Richter D., and Swedo S. E. (1996) Case study: acute basal ganglia enlargement and obsessive-compulsive symptoms in an adolescent boy. *J. Am. Acad. Child Adolesc. Psychiatry* 35: 913-915

82. Allen A. J., Leonard H. L., and Swedo S. E. (1995) Case study: a new infection-triggered, autoimmune subtype of pediatric OCD and Tourette's syndrome. *J. Am. Acad. Child Adolesc. Psychiatry* 34: 307-311

83. Muller N., Riedel M., Erfurth A., and Moller H. J. (1997) Immunoglobulin therapy in Gilles de la Tourette syndrome. *Nervenarzt* 68, 914-916.

84. Perlmutter S. J., Garvey M. A., Castellanos X., Mittleman B. B., Giedd J., Rapoport J. L. et al. (1998) A case of pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections. *Am. J. Psychiatry* 155: 1592-1598

85. Swedo S. E., Leonard H. L., Garvey M., Mittleman B., Allen A. J., Perlmutter S. et al. (1998) Pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections: clinical description of the first 50 cases [published erratum appears in *Am J Psychiatry* 1998 Apr;155(4):578]. Am. J. Psychiatry 155: 264-271

86. Swedo S. E., Leonard H. L., Schapiro M. B., Casey B. J., Mannheim G. B., Lenane M. C. et al. (1993) Sydenham's chorea: physical and psychological symptoms of St Vitus dance. *Pediatrics* 91: 706-713

87. Kiessling L. S., Marcotte A. C., and Culpepper L. (1993) Antineuronal antibodies in movement disorders. *Pediatrics* 92: 39-43

88. Swedo S. E., Leonard H. L., Mittleman B. B., Allen A. J., Rapoport J. L., Dow S. P. et al. (1997) Identification of children with pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections by a marker associated with rheumatic fever. *Am. J. Psychiatry* 154: 110-112

89. Swedo S. E. (1994) Sydenham's chorea. A model for childhood autoimmune neuropsychiatric disorders. *JAMA* 272: 1788-1791

90. Kurlan R. (1998) Tourette's syndrome and 'PANDAS': will the relation bear out? Pediatric autoimmune neuropsychiatric disorders associated with streptococcal infection. *Neurology* 50: 1530-1534

91. Shulman S. T. (1999) Pediatric autoimmune neuropsychiatric disorders associated with streptococci (PANDAS). *Pediatr. Infect. Dis*. J. 18: 281-282

92. Hoekstra P.J ., Kallenberg C. G., Korf J., and Minderaa R. B. (2002) Is Tourette's syndrome an autoimmune disease? *Mol. Psychiatry* 7: 437-445

93. Singer H. S. and Loiselle C. (2003) PANDAS. A commentary. *J. Psychosom. Res*. 55: 31-39

94. Garvey M. A., Perlmutter S. J., Allen A. J., Hamburger S., Lougee L., Leonard H. L. et al. (1999) A pilot study of penicillin prophylaxis for neuropsychiatric exacerbations triggered by streptococcal infections. *Biol. Psychiatry* 45: 1564-1571

95. Murphy M. L. and Pichichero M. E. (2002) Prospective identification and treatment of children with pediatric

autoimmune neuropsychiatric disorder associated with group A streptococcal infection (PANDAS). Arch. Pediatr. Adolesc. Med. 156: 356-361

96. Muller N., Riedel M., Forderreuther S., Blendinger C., and Abele-Horn M. (2000) Tourette's syndrome and mycoplasma pneumoniae infection. *Am. J. Psychiatry* 157: 481-482

97. Hoekstra P. J., Manson W. L., Steenhuis M. P., Kallenberg C. G., and Minderaa R. B. Association of common cold with exacerbations in tic disorder patients: a prospective longitudinal study. *Eur. Child Adolesc. Psychiatry* [Abstract] (in press).

98. Cardona F. and Orefici G. (2001) Group A streptococcal infections and tic disorders in an Italian pediatric population. *J. Pediatr.* 138: 71-75

99. Giulino L., Gammon P., Sullivan K., Franklin M., Foa E., Maid R., et al. (2002) Is parental report of upper respiratory infection at the onset of obsessive-compulsive disorder suggestive of pediatric autoimmune neuropsychiatric disorder associated with streptococcal infection? J. Child Adolesc. *Psychopharmacol*. 12: 157-164

100. Muller N., Kroll B., Schwarz M. J., Riedel M., Straube A., Lutticken R. et al. (2001) Increased titers of antibodies against streptococcal M12 and M19 proteins in patients with Tourette's syndrome. *Psychiatry Res*. 101: 187-193

101. Muller N., Riedel M., Straube A., Gunther W., and Wilske B. (2000) Increased anti-streptococcal antibodies in patients with Tourette's syndrome. *Psychiatry Res.* 94: 43-49

102. Church A. J., Dale R. C., Lees A. J., Giovannoni G., and Robertson M. M. (2003) Tourette's syndrome: a cross sectional study to examine the PANDAS hypothesis. *J. Neurol. Neurosurg. Psychiatry* 74: 602-607

103. Morshed S. A., Parveen S., Leckman J. F., Mercadante M. T., Bittencourt Kiss M. H., Miguel E. C. et al. (2002) Antibodies against neural, nuclear, cytoskeletal and streptococcal epitopes in children and adults with Tourette's syndrome, Sydenham's chorea, and autoimmune disorders. Biol. Psychiatry 50: 566-577

104. Singer H. S., Giuliano J. D., Hansen B. H., Hallett J. J., Laurino J. P., Benson M. et al. (1998) Antibodies against human putamen in children with Tourette syndrome. *Neurology* 50: 1618-1624

105. Peterson B. S., Leckman J. F., Tucker D., Scahill L., Staib L., Zhang H. et al. (2000) Preliminary findings of antistreptococcal antibody titers and basal ganglia volumes in tic, obsessive-compulsive, and attention deficit/hyperactivity disorders. *Arch. Gen. Psychiatry* 57: 364-372

106. Loiselle C. R., Wendlandt J. T., Rohde C. A., and Singer H. S. (2003) Antistreptococcal, neuronal, and nuclear antibodies in Tourette syndrome. *Pediatr. Neurol*. 28: 119-125

107. Khanna A. K., Buskirk D. R., Williams R. C. Jr., Gibofsky A., Crow M. K., Menon A. et al. (1989) Presence of a non-HLA B cell antigen in rheumatic fever patients and their families as defined by a monoclonal antibody. *J. Clin. Invest* 83: 1710-1716

108. Taneja V., Mehra N. K., Reddy K. S., Narula J., Tandon R., Vaidya M. C. et al. (1989) HLA-DR/DQ antigens and reactivity to B cell alloantigen D8/17 in Indian patients with rheumatic heart disease. *Circulation* 80: 335-340

109. Murphy T. K., Goodman W. K., Fudge M. W., Williams R. C. Jr., Ayoub E. M., Dalal M. et al. (1997) B lymphocyte antigen D8/17: a peripheral marker for childhood-onset obsessive-compulsive disorder and Tourette's syndrome? *Am. J. Psychiatry* 154: 402-407

110. Hollander E., Delgiudice-Asch G., Simon L., Schmeidler J., Cartwright C., Decaria C. M. et al. (1999) B lymphocyte antigen D8/17 and repetitive behaviors in autism. *Am. J. Psychiatry* 156: 317-320

111. Niehaus D. J., Knowles J. A., van Kradenberg J., du Toit W., Kaminer D., Seedat S. et al. (1999) D8/17 in obsessive-compulsive disorder and trichotillomania. *S. Afr. Med. J.* 89: 755-756

112. Sokol M. S., Ward P. E., Tamiya H., Kondo D. G., Houston D., and Zabriskie J. B. (2002) D8/17 expression on B lymphocytes in anorexia nervosa. *Am. J. Psychiatry* 159: 1430-1432

113. Chapman F., Visvanathan K., Carreno-Manjarrez R., and Zabriskie J. B. (1998) A flow cytometric assay for D8/17 B cell marker in patients with

Tourette's syndrome and obsessive compulsive disorder. *J. Immunol. Methods* 219:181-186

114. Hoekstra P. J., Bijzet J., Limburg P. C., Steenhuis M. P., Troost P. W., Oosterhoff M. D. et al. (2001) Elevated D8/17 expression on B lymphocytes, a marker of rheumatic fever, measured with flow cytometry in tic disorder patients. *Am. J. Psychiatry* 158: 605-610

115. Murphy T. K., Benson N., Zaytoun A., Yang M., Braylan R., Ayoub E. et al. (2001) Progress toward analysis of D8/17 binding to B cells in children with obsessive compulsive disorder and/or chronic tic disorder. *J. Neuroimmunol*. 120: 146-151

116. Hamilton C. S., Garvey M. A., and Swedo S. E. (2003) Sensitivity of the D8/17 assay. *Am. J. Psychiatry* 160: 1193-1194

117. Eisen J. L., Leonard H. L., Swedo S. E., Price L. H., Zabriskie J. B., Chiang S. Y. et al. (2001) The use of antibody D8/17 to identify B cells in adults with obsessive-compulsive disorder. *Psychiatry Res.* 104: 221-225

118. Inoff-Germain G., Rodriguez R. S., Torres-Alcantara S., Diaz-Jimenez M. J., Swedo S. E., and Rapoport J. L. (2003) An immunological marker (D8/17) associated with rheumatic fever as a predictor of childhood psychiatric disorders in a community sample. *J. Child Psychol. Psychiatry* 44: 782-790

119. Husby G., van de Rijn I, Zabriskie J. B., Abdin Z. H., and Williams R. C. Jr. (1976) Antibodies reacting with cytoplasm of subthalamic and caudate nuclei neurons in chorea and acute rheumatic fever. *J. Exp. Med*. 144: 1094-1110

120. Laurino J. P., Hallett J., Kiessling L. S., Benson M., Pelletier T., and Kuhn C. (1997) An immunoassay for anti-neuronal antibodies associated with involuntary repetitive movement disorders. *Ann. Clin. Lab Sci.* 27: 230-235

121. Singer H. S., Giuliano J. D., Hansen B. H., Hallett J. J., Laurino J. P., Benson M. et al. (1999) Antibodies against a neuron-like (HTB-10 neuroblastoma) cell in children with Tourette syndrome. *Biol. Psychiatry* 46: 775-780

122. Perlmutter S. J., Leitman S. F., Garvey M. A., Hamburger S., Feldman E., Leonard H. L. et al. (1999) Therapeutic plasma exchange and intravenous immunoglobulin for obsessive-compulsive disorder and tic disorders in childhood. *Lancet* 354: 1153-1158

123. Hallett J. J., Harling-Berg C. J., Knopf P. M., Stopa E. G., and Kiessling L. S. (2000) Anti-striatal antibodies in tourette syndrome cause neuronal dysfunction [In Process Citation]. *J. Neuroimmunol*. 111: 195-202

124. Taylor J. R., Morshed S. A., Parveen S., Mercadante M. T., Scahill L., Peterson B. S. et al. (2002) An animal model of Tourette's syndrome. *Am. J. Psychiatry* 159: 657-660

125. Wendlandt J. T., Grus F. H., Hansen B. H., and Singer H. S. (2001) Striatal antibodies in children with Tourette's syndrome: multivariate discriminant analysis of IgG repertoires. *J. Neuroimmunol*. 119: 106-113

126. Hoekstra P. J., Horst G., Limburg P. C., Troost P. W., van Lang N., de Bildt A. et al. (2003) Increased seroreactivity in tic disorder patients to a 60 kDa protein band from a neuronal cell line. *J. Neuroimmunol*. (in press).

127. Bukau B. and Horwich A. L. (1998) The Hsp70 and Hsp60 chaperone machines. *Cell* 92: 351-366

128. Wick G., Perschinka H., and Millonig G. (2001) Atherosclerosis as an autoimmune disease: an update. *Trends Immunol*. 22: 665-669

129. Multhoff G., Botzler C., and Issels R. (1998) The role of heat shock proteins in the stimulation of an immune response. *Biol. Chem*. 379: 295-300

130. van Roon J. A., van Eden W., van Roy J. L., Lafeber F. J., and Bijlsma J. W. (1997) Stimulation of suppressive T cell responses by human but not bacterial 60-kD heat-shock protein in synovial fluid of patients with rheumatoid arthritis. *J. Clin. Invest* 100: 459-463

131. Yokota S. I., Hirata D., Minota S., Higashiyama T., Kurimoto M., Yanagi H. et al. (2000) Autoantibodies against chaperonin CCT in human sera with rheumatic autoimmune diseases: comparison with antibodies against other Hsp60 family proteins. *Cell Stress. Chaperones*. 5: 337-346

132. Zhu J., Quyyumi A. A., Rott D., Csako G., Wu H., Halcox J. et al. (2001) Antibodies to human heat-shock protein 60 are associated with the presence and severity of coronary artery disease: evidence for an autoimmune component of atherogenesis. *Circulation* 103: 1071-1075

133. Lougee L., Perlmutter S. J., Nicolson R., Garvey M. A., and Swedo S. E. (2000) Psychiatric disorders in first-degree relatives of children with pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections (PANDAS). *J. Am. Acad. Child Adolesc. Psychiatry* 39: 1120-1126

Concluding remarks

Concluding remarks

To classify a disease as autoimmune, one must show that an adaptive immune response to a self-antigen causes the observed pathology. One mechanism of autoimmunity is through autoantibody binding to autoantigens. Do the results of the experiments described in this thesis provide confirming evidence to classify Tourette's syndrome as an autoimmune disease? The answer should be, at the most, not yet. In tic disorder patients, we did detect increased frequency in serum of autoantibodies directed against autoantigens, but did, as yet, not establish a role for the autoantibody-autoantigen interaction in the disease process. Also, we identified differences between tic disorder patients and healthy controls with regard to B lymphocyte surface antigens, but do not know the functional significance of this finding. Moreover, we provided evidence for an association between common, viral upper respiratory infections and subsequent exacerbations of tic severity in children, but have no clue with regard to the immunological pathway that may be involved. Infections are known to induce flare-ups in certain autoimmune conditions, such as Wegener's granulomatosis.[1]

As outlined in chapter 11, our findings are best summarized as pointing to a rather general activation of the immune system in patients with a tic disorder. Thus, even though the precise mechanism is largely unknown, at present, the available evidence from our experiments and those of others does seem to suggest that immune factors appear to be involved in tic disorders. Two findings in the present thesis do not provide evidence for the involvement of the immune system in tic disorders. First, we found no indication that the metabolism of tryptophan through the kynurenine pathway would be altered in tic disorder patients. Thus, these results could not confirm a more than usual activation of indoleamine 2,3-dioxygenase through pro-inflammatory cytokines, which would promote the breakdown of tryptophan to kynurenine. Furthermore, intravenously administered immunoglobulins did not appear to be an effective treatment in patients with a tic disorder. Positive results would have been an indirect clue to a possible pathogenic role of autoantibodies in tic disorders. Our longitudinal study regarding the role of psychosocial stress, however, is well compatible with the autoimmune hypothesis of Tourette's syndrome. The results of this study indicate that in about 20% of individuals with a tic disorder, the changing tic severity over time appears to be associated with small life events. In many autoimmune conditions, psychosocial stress may adversely influence disease severity.[2]

What type of studies do we need to get more definite answers to the presumed involvement of autoimmunity in tic disorders? Relevant antigenic structures should be characterized at a molecular level. This regards both putative susceptibility markers on B lymphocytes and autoantigenic targets of autoantibodies with relevance for the pathogenesis of Tourette's syndrome. Results could lead to the development of animal models in which the functional significance of identified immune alterations could be investigated *in vivo*. In addition, longitudinal studies should study the involvement of immune factors,

including B lymphocyte subsets, autoantibody-autoantigen interactions, and intercurrent infections in relation to the onset, changing severity pattern, and possible remission of the tics over the course of time. Finally, identification of major genes involved in Tourette's syndrome would greatly enhance such studies.

References

1. Pinching, A.J., Rees, A.J., Russell, B.A., Lockwood, C.M., Mitchison, R.S., and Peters, D.K.: Relapses in Wegener's granulomatosis: the role of infection. *BMJ* 1980, 281:836-838.

2. O'Leary A.: Stress, emotion and human immune function. *Psychol Bull*. 1990, 108:363-382.

Summary

Summary

Gilles de la Tourette's syndrome is a childhood-onset neuropsychiatric disorder characterized by the presence of both multiple muscle movements and vocalizations, referred to as motor and vocal tics. Some research findings suggest a central role for the involvement of autoimmunity in the pathogenesis of tic disorders. These seem to suggest that infections may induce or reinforce tics and associated features in susceptible individuals, possibly through the involvement of abnormal humoral immune responses directed against self-tissue antigens. This possible role of immune dysregulations in the pathogenesis of tic disorders is the topic of the present thesis.

In *chapter 1*, we provide a review of research findings which may support the involvement of autoimmunity in childhood-onset tic disorders, in particular the presence of antineuronal autoantibodies, D8/17 B lymphocyte overexpression, a marker of chorea associated with streptococcal infection, and possible beneficial effects of immunomodulatory intervention. One of the most controversial areas in this field is the validity of the proposed PANDAS concept. Some researchers have delineated a putatively unique subgroup of patients from the spectrum of illness encompassing Tourette's syndrome and obsessive-compulsive disorder (OCD), whose tics and obsessive-compulsive symptoms are shown to arise in response to beta-hemolytic streptococcal infections. They designated it by the term pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections (PANDAS). Herein we, additionally, present pros and cons concerning the concept of PANDAS. Finally, recommendations for future research directions are given.

Chapter 2 describes a study in which we investigated social and behavioral problems related to attention deficit/hyperactivity disorder (ADHD), severity of obsessions and compulsions, and tic severity in children with a tic disorder. Parents of 58 clinically referred children with a tic disorder with and without different forms of ADHD filled out the Child Behavior Checklist (CBCL) and the Children's Social Behavior Questionnaire, a novel parent questionnaire covering a broad range of behavior problems as seen in children with milder variants of pervasive developmental disorders. Comparisons were made between tic disorder patients with primarily hyperactive-impulsive ADHD, tic disorder patients with primarily inattentive ADHD, and tic disorder patients without ADHD. Also, part correlations of subscale scores of the questionnaires with measures of ADHD severity, severity of obsessions and compulsions, and tic severity, respectively, were executed, while controlling for the predictive value of the other two measures on the outcome of the questionnaires. Results indicated that tic disorder patients with primarily hyperactive-impulsive ADHD had the highest scores on the parent questionnaires, tic disorder patients with primarily inattentive ADHD medium scores, and tic disorder patients without ADHD the lowest. On a considerable number of subscales, significant part correlations with ADHD severity, but not tic severity were obtained. Severity of obsessions and compulsions was independently

correlated with the CBCL subscale thought problems, but not with most other subscales. There was no significant correlation between tic severity and ADHD severity. In conclusion, in patients with a tic disorder it is the presence and severity of ADHD that is the main predictor of associated behavior and social problems.

The purpose of the study described in *chapter 3* was to assess D8/17 expression on B lymphocytes of tic disorder patients by using an objective method in which no operator variability was involved. Elevated D8/17 expression on B lymphocytes is a known susceptibility marker of rheumatic fever. Previous studies had also reported increased D8/17 expression on B lymphocytes of patients with tic disorders. In our study, D8/17 expression on B lymphocytes was assessed with flow cytometry by using an IgM monoclonal D8/17-specific antibody in an unselected group of Dutch patients with tic disorders (N=33) and healthy volunteers (N=20). Binding of this monoclonal antibody was compared with binding of an irrelevant IgM monoclonal antibody and the shift in mean fluorescence intensity of the D8/17-specific antibody compared to that of the irrelevant IgM monoclonal antibody was used as a measure of D8/17 overexpression. For the patients, Yale Global Tic Severity Scale scores were used to assess disease severity. We found that D8/17 overexpression in the patient group (mean=16.8 arbitrary units, SD=30.5) was significantly higher than in the control group (median=3.2, SD=3.0). A significant minority of the patients (N=13, 39.4%), however, had levels of D8/17 overexpression within the range of that of the healthy comparison subjects. Flow cytometric analysis did not indicate a separate subpopulation of D8/17 positive B cells. These data confirm the utility of D8/17 B cell overexpression as a peripheral blood marker in patients with tic disorders and are compatible with a streptococcus-related pathogenesis for at least a subgroup of patients with tic disorders.

However, in *chapter 4*, we present a reanalysis and reinterpretation of the results of the preceding study. At reanalysis of the data, there appeared to be an unexpected, close correlationship between the mean fluorescence intensity produced by the irrelevant IgM, MOC32, and that by the D8/17-specific IgM, in both patients and controls. This could suggest that, at least in part, we did not detect D8/17 overexpression on B cells in tic disorder patients compared to healthy controls, but rather, increased expression of receptors for the constant parts of IgM molecules (Fc-μ) on B cells, so explaining increased binding of both the D8/17-specific monoclonal antibody and the irrelevant monoclonal antibody (MOC32), possibly due to a more general state of immune activation.

Chapter 5 contains the results of a study on D8/17 B lymphocyte expression in a small group of Dutch patients with post-streptococcal reactive arthritis. Only 29% of patients appeared to have an elevated percentage of D8/17 positive B lymphocytes, which is in contrast with the 63%-100% in the acute rheumatic fever literature. This may suggest non-susceptiblity to developing acute rheumatic fever in the majority of Dutch post-streptococcal reactive arthritis patients.

In tic disorders, increased seroreactivity against neuronal antigens had been demonstrated, without performing molecular characterization of antigens. In *chapter 6*, unselected patients with a tic disorder were compared with healthy

controls, patients with autistic disorder (AD), and persons with OCD. Seroreactivity against neuroblastoma cells was analyzed by Western blot. Binding to a 60 kD protein occurred significantly more frequently in tic disorder patients (67.1%) than in AD patients (40.0%), OCD patients (40.0%), and controls (41.9%). Sequence analysis identified this as a heat shock protein, a protein not exclusive to neuronal tissue. This may support the involvement of autoimmunity in tic disorders.

Increased levels of plasma kynurenine have been reported in tic disorder patients and this observation has been suggested to be indicative of immune dysregulation in tic disorders. In *chapter 7*, we aimed to replicate this finding in a large group of well-characterized tic disorder patients. Plasma concentrations of tryptophan and kynurenine were determined in Dutch patients with Tourette's disorder (N=44), patients with chronic motor tic disorder (N=15), and healthy volunteers (N=32). Correlations between Yale Global Tic Severity Scale scores and the plasma levels were examined. Our results revealed no significant differences between patients and controls in plasma tryptophan and kynurenine concentrations or in the kynurenine to tryptophan ratio. Also, kynurenine did not correlate with indices of tic severity. A small, but statistically significant negative relationship ($r=-0.341$; $p=0.009$) was found between tryptophan levels and vocal, but not motor tic severity. These results indicate that the metabolism of tryptophan through the kynurenine pathway is not altered in tic disorders. Although it is unclear how much may be inferred about immune function based on measurement of plasma kynurenine, the results do not lend support to hypothesized alterations in immune processes in tic disorder patients.

Cross-sectional data and some case studies suggest a temporal relationship between fluctuations in tic severity and preceding infections. The aim of the study described in *chapter 8* was to examine this possible relationship in a prospective longitudinal design. Two groups of tic disorder patients were included in this study, a pediatric group between 7 and 15 years (N=20) and an adult group of 16 years and older (N=41). During a 24 weeks' period, participants were asked to weekly fill out self-questionnaires regarding the presence of tic exacerbations and experience of common cold. In addition, six throat swabs were taken at fixed intervals irrespective of symptoms, and cultured for streptococci; also, three serial serum assessments of streptococcal antibodies were performed. In the pediatric group, the self-report of a common cold was strongly associated with an exacerbation in tic severity 4 weeks later (odds ratio=4.685, $p=0.001$). In this group, no association between the self-report of a common cold and tic exacerbations was found in other weeks. In the adult group, we found no association at all between reports of common cold and tic exacerbations. Association with streptococcal infections could not be determined due to the limited number of observed streptococcal infections. In conclusion, while it remains to be proven whether or not streptococcal infections are associated with exacerbations in tic severity, this study points to a hitherto unknown association of common viral infections with exacerbations in tic severity in children, which may support the involvement of immune dysregulation in tic disorders.

Clinical experience suggests an association between stressful life events and fluctuations in symptom severity of tic disorder patients. The aim of the study in *chapter 9* was to examine this possible relationship in a prospective longitudinal design. Two groups of tic disorder patients were included in this study, a pediatric group between 7 and 16 years (N=25; 24 of whom completed the study) and an adult group of 18 years and older (N=32; 28 of whom completed the study, and reported at least one life event). During a 12 weeks' period, participants were asked to weekly fill out self-questionnaires regarding the occurrence of small life events and self-rating of tic severity. In the adult group as a whole, we found a weak but statistically significant correlation between negative small life events and tic severity during the same week ($r=0.268$, $p<0.001$). However, only a minority of individual pediatric (21%) and adult patients (18%) demonstrated significant relationships between the frequency of small life events and tic severity in the same week or 1 week later, with undesirable small life events positively associated with tic severity in some patients and negatively associated with tic severity in other patients. We concluded that, contrary to traditional views, in general, life events do not account for changes in tic severity. Only in a minority of tic disorder patients, fluctuations in symptom severity appear to be associated with possibly stressful small life events.

Chapter 10 describes the results of a double-blind placebo-controlled study regarding the efficacy of intravenously administered immunoglobulins (IVIG) in patients with a tic disorder. Case studies and a placebo-controlled study had previously suggested the effectiveness of immunomodulatory therapy in those patients with tic or related disorders whose symptoms show a relationship with streptococcal infections. No studies had been conducted on the effectiveness of IVIG on tic severity in unselected tic disorder patients. In our study, 30 tic disorder patients were randomized to IVIG (1 g/kg on two consecutive days) or placebo. Symptom rating occurred at baseline and at week two, four, six, 10, and 14 post-treatment, after which blinding was broken. We observed no significant differences between both treatment groups regarding post-treatment changes in tic severity. Severity of obsessions and compulsions, which was in the subclinical range at base line, decreased significantly in the IVIG group compared to the placebo group at week six ($p=0.02$). Then, there was a 32.3% improvement in the IVIG group compared to base line level. Though this improvement was maintained over the following 8 weeks, no statistically significant differences between the IVIG and the placebo group with regard to improvements in obsessions and compulsions were detected at subsequent assessments. Thus, based on these results, IVIG cannot be recommended in tic disorders.

In *chapter 11*, we present an overall discussion with regard to the relevance of immune factors in tic disorders, taking into account the various results of the experiments described in this thesis. In this chapter, we start with a general overview of the neurobiology of Tourette's syndrome with regard to neuroimaging, neurochemistry and genetics. While the pathogenesis at a molecular and cellular level remains unknown, structural and functional neuroimaging studies point to the involvement of the basal ganglia and related

cortico-striato-thalamo-cortical circuits as the neuroanatomical site for Tourette's syndrome. Evidence is also available for abnormalities within the dopaminergic neurotransmitter system. Moreover, Tourette's syndrome has a strong genetic background, and considerable progress has been made in identifying possibly involved genomic loci. We conclude that Tourette's syndrome is a heterogeneous disorder, and that immune factors may indeed be involved in some patients.

Samenvatting in het Nederlands

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Ticstoornissen waaronder het syndroom van Gilles de la Tourette worden gekenmerkt door herhaald optredende onwillekeurige spierbewegingen en geluiden, zogenaamde motorische en vocale tics. Tics beginnen over het algemeen op de kinderleeftijd, gemiddeld op het zevende levensjaar. We weten niet precies waardoor tics ontstaan. Wel weten we dat erfelijke factoren in sterke mate betrokken zijn. Daarnaast zijn er aanwijzingen dat infecties gepaard gaand met keelpijn, veroorzaakt door een bepaalde bacterie (de streptokok) een rol zouden kunnen spelen in het ontstaan of het verergeren van tics. Sommige bevindingen lijken erop te wijzen dat auto-immuniteit hierbij betrokken is. Men spreekt van auto-immuniteit als de normale afweer van het lichaam zich niet meer uitsluitend richt op ziekte-indringers, zoals bacteriën en virussen, maar ook op onderdelen van het eigen lichaam. In het geval van ticstoornissen wordt er daarbij gedacht aan afweerreacties tegen bepaalde hersengebieden die vervolgens tot tics aanleiding zouden kunnen geven. Hierbij spelen mogelijk auto-antilichamen een rol. Dit zijn stoffen die zich richten tegen lichaamseigen bestanddelen en soms schade kunnen veroorzaken.

In *hoofdstuk 1* geven we een algemeen overzicht van bestaande onderzoeksbevindingen die zouden kunnen passen bij de betrokkenheid van auto-immuniteit bij ticstoornissen. We gaan hierbij allereerst in op onderzoeken die wijzen op de aanwezigheid van auto-antilichamen tegen hersenweefsel in het bloed van mensen met tics. Daarna beschrijven we onderzoeken die hebben gekeken naar de verhoogde aanwezigheid van een bepaald eiwit op bepaalde bij de afweer betrokken witte bloedlichaampjes, namelijk het eiwit D8/17 op B-lymfocyten (bloedcellen die bij de afweer tegen bacteriën betrokken zijn). Eerder was verhoogde aanwezigheid van D8/17 op B-lymfocyten gezien bij patiënten met acuut reuma, een ziektebeeld als gevolg van auto-immuun reacties in aansluiting aan een streptokokkeninfectie, een infectie die normaal alleen gekenmerkt wordt door keelpijn. Verhoogde aanwezigheid van D8/17 op B-lymfocyten bij patiënten met tics zou dus een indirecte aanwijzing voor auto-immuniteit in aansluiting aan een streptokokkeninfectie kunnen zijn. Verder geven we een overzicht van onderzoeken waarin gerapporteerd is over het mogelijk gunstige effect van behandelingen die ingrijpen op het immuunsysteem bij personen met tics.

Hoofdstuk 2 beschrijft een onderzoek dat geen betrekking heeft op de rol van auto-immuniteit. Daarentegen bespreken we een studie naar de aanwezigheid van gedragsproblemen en problemen in de omgang met andere kinderen bij patiënten met tics met en zonder aandachtstekortstoornis met hyperactiviteit (ADHD). Hierbij werd aan de hand van oudervragenlijsten die onder andere sociaal gedrag meten (bijvoorbeeld hoe kinderen bij hun gedrag wel of niet rekening houden met anderen), gekeken of er verschillen bestaan ten opzichte van leeftijdsgenoten zonder tics en of die verschillen meer met de tics samenhangen of meer met de ernst van de ADHD. Het onderzoek laat zien dat de gedrags- en sociale

problemen bij kinderen met tics voornamelijk in verband staan met ADHD en niet met tics als zodanig. De belangrijkste conclusie van dit deel van het onderzoek is, dat niet het hebben van tics, maar de eventuele bijkomende ADHD gepaard gaat met problemen in het begrijpen en toepassen van sociale informatie.

Het onderzoek in *hoofdstuk 3* is het eerste onderzoek op het terrein van de immunologie binnen dit proefschrift. We hebben de mate van aanwezigheid van het eerder vermelde D8/17 eiwit op B-lymfocyten gemeten bij zowel patiënten met tics als bij gezonde proefpersonen. In vergelijking met eerdere onderzoeken hebben we hierbij een nieuwe, sterk verbeterde methode gebruikt. Eerdere studies hadden gebruik gemaakt van subjectieve handmatige tellingen van cellen, terwijl wij een objectieve, volledig geautomatiseerde meting toepasten. In ons onderzoek vonden we inderdaad aanwijzingen voor een grotere aanwezigheid van het eiwit D8/17 op B-lymfocyten bij de meeste patiënten met tics in vergelijking met de gezonde proefpersonen. De precieze betekenis van deze bevinding is onduidelijk. Het wijst mogelijk op een betrokkenheid van het afweersysteem bij de aanwezigheid van tics.

In *hoofdstuk 4* geven we echter een alternatieve interpretatie van de bevindingen van het onderzoek van hoofdstuk 3. Mogelijk is bij patiënten met tics niet zozeer het oppervlakte eiwit D8/17 in verhoogde mate aanwezig op B-lymfocyten, maar receptoren die een bepaald type antilichamen in het algemeen binden. Met een antilichaam dat aan D8/17 bindt, proberen we een beeld te krijgen van de hoeveelheid D8/17 aan de buitenkant van de B-lymfocyt; dat antichaam bindt echter ook aan het betreffende type receptoren die eveneens aan de buitenkant van B-lymfocyten aanwezig zijn en die bij activatie van de B-lymfocyt in sterkere mate aanwezig kunnen zijn. De verhoogde binding bij patiënten met tics zou kunnen wijzen op algemene activatie van het immuunsysteem. We weten dus niet precies wat gemeten wordt bij onze meetmethode gericht op de mate van aanwezigheid van D8/17.

De boven aangegeven meetmethode om de hoeveelheid D8/17 aan de buitenkant van B-lymfocyten te meten hebben we in *hoofdstuk 5* ook toegepast bij een andere groep patiënten, namelijk patiënten met gewrichtsontstekingen in aansluiting aan een streptokokkeninfectie. Bij dit ziektebeeld wordt aangenomen dat auto-immuniteit waarschijnlijk een rol speelt. Het bleek echter dat maar een minderheid van de patiënten een verhoogde mate van D8/17 op de B-lymfocyten had (dan wel een verhoogde mate van de eerder genoemde receptoren). Kennelijk is de betrokkenheid van het afweersysteem bij deze gewrichtsontstekingen anders dan mogelijk bij tics het geval is.

Verder keken we in het onderzoek dat beschreven staat in *hoofdstuk 6* in het bloed naar auto-antichamen tegen hersenweefsel. We vonden dat personen met tics vaker reageerden met een bepaald eiwit dat ook, maar niet alleen, in de hersenen voorkomt. Dit eiwit is een zogenaamd heat-shock eiwit. Dit eiwit maakt ook deel uit van veel bacteriën en virussen. Mogelijk wijzen onze bevindingen erop dat infecties een rol spelen bij tics. Ook is het aanwezig zijn van de auto-antilichamen een aanwijzing voor het betrokken zijn van auto-immuniteit bij ticstoornissen.

In *hoofdstuk 7* staat onderzoek beschreven naar de stof kynurenine in het bloed bij personen met tics vergeleken met gezonde personen. Verhoogde concentraties kynurenine wijzen mogelijk op betrokkenheid van de afweer. In dit opzicht vonden we echter geen verschillen tussen personen met tics en gezonde personen.

In het onderzoek in *hoofdstuk 8* vroegen we patiënten met tics gedurende een half jaar om elke week schriftelijk aan ons door te geven, hoe het met de ernst van de tics stond in vergelijking met een week eerder en ook of er sprake was van een doorgemaakte verkoudheid. Er bleek, althans bij kinderen, een duidelijk verband tussen het doormaken van een verkoudheid en een daaropvolgende verergering van de tics precies vier weken later. Kennelijk is het doormaken van een verkoudheid een belangrijke risicofactor voor een verergering van de tics vier weken later. Bij volwassenen kwam dit verband er niet uit, al waren er ook in deze groep aanwijzingen voor een verband, maar dan drie weken later. Ook deze bevindingen wijzen, net als de bevinding van hoofdstuk 6, op de rol die infecties spelen bij tics. Uit ons onderzoek blijkt bovendien dat het in tegenstelling tot wat eerder werd verondersteld, in dit verband zeker niet om uitsluitend streptokokkeninfecties gaat. Verkoudheden worden juist bijna altijd door virussen veroorzaakt.

Ook keken we, in *hoofdstuk 9*, in wekelijkse vragenlijsten naar een verband met stress en verergeringen van de tics. Hieruit is gebleken dat maar bij 20% van de personen met tics er een verband was tussen doorgemaakte stressvolle gebeurtenissen en wisselingen in ernst van de tics. Bij de rest van de personen was dat verband er niet. Kennelijk speelt stress maar bij een minderheid van de mensen met tics een rol in het erger worden van de tics. Overigens kunnen stressvolle gebeurtenissen soms ook een rol spelen bij een verergering van auto-immuunziekten.

Tot slot onderzochten we zoals beschreven staat in *hoofdstuk 10*, in hoeverre het via een infuus toedienen van immuunglobulinen een goede behandeling zou zijn voor de tics. Hiertoe hebben veertien mensen via het infuus gedurende twee dagen een nepmiddel (placebo) gekregen en vijftien mensen de echte immuunglobulinen. Helaas bleek dat het niets uitmaakte of er nu het placebo middel of de immuunglobulinen gegeven werden. De tics bleven gemiddeld gelijk in ernst. Wel was het zo dat de dwangklachten gemiddeld een lichte afname vertoonden bij degenen die de immuunglobulinen hadden gekregen. De conclusie moet echter zijn dat immuunglobulinen geen goede behandeling zijn bij het syndroom van Gilles de la Tourette.

In *hoofdstuk 11* hebben we in een overzichtsartikel onze bevindingen en die van anderen samengevat om een voorlopig slotantwoord te vinden op de vraag in hoeverre auto-immuniteit en infecties betrokken zijn bij ticstoornissen. Onze belangrijkste conclusie luidt dat het te vroeg is om te concluderen dat het syndroom van Gilles de la Tourette een auto-immuunziekte is, maar dat er wel belangrijke aanwijzingen zijn dat het immuunsysteem en infecties een rol spelen in het ziektebeeld.

Dankwoord

Dankwoord

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Andrea heeft, zoals dat heet, alle pieken en dalen van het onderzoek van nabij meegemaakt. Haar rol was echter veel groter dan een van emotionele steun. Zonder dat ze het zelf altijd door had, heeft ze mij op een aantal uiterst cruciale punten in het onderzoek op de juiste aanpak gewezen. Verder was het mij alleen nooit gelukt, het boekje zo mooi vorm te geven. Heel veel dank voor dit alles! Ik hoop dat ik ook iets kan betekenen voor haar eigen proefschrift.